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Calling Attention To 1035

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CHAUNCEY D. LEAKE

DOCTOR OF PHILOSOPHY

A brochure of appreciation including a pen sketch of Dr. Leake was prepared by a Special Resolutions Committee: Miss Virginia Dryden, Mr. George Crosby, Dr. Frank Engley, Dr. Raymond Gregory, Dr. Titus Harris, Mr. E. F. Kinzer, Dr. Robert Moore, Dr. Charles Pomerat, Dr. Theodore Panos, Dr. John Sinclair, and Dr. Howard Swann. It was announced by the faculty of the Medical Branch, in Galveston, April 5, 1955, and was widely distributed.

Texas Reports on Biology and Medicine was founded in 1943 as a quarterly journal for the publication of research from the laboratories not only of the Medical Branch but also from other parts of the system of higher education in the State. Doctor Leake placed the item in the Library budget because the *Reports* were to serve as the basis of requests for exchange with other publications. As a result the Library now receives approximately 700 such journals by exchange. The present number completes the thirteenth volume with a total of about 8000 pages. Four thousand copies are printed and of these 900 are sent abroad reaching all medical libraries in the world. Doctor Leake has served as Editor for the entire period. During this period he has written and published the variety of articles which we append here to indicate the versatility of his activity.

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While Executive Director of

The University of Texas Medical Branch

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In addition, thirteen volumes of *Texas Reports on Biology and Medicine* were edited and monthly notes were made "Calling Attention To" items of interest.

THE PERSISTENCE (SURVIVAL) OF MICROORGANISMS: I. AIR-BORNE ORGANISMS

FRANK B. ENGLEY, JR.*

Introduction

Considerable interest has arisen since the development of microbiology to determine the survival or death of organisms under the influence of nature, as well as under physical factors under laboratory conditions. The reports of these studies are scattered throughout biological reports. They have never been collected in a survey in an effort to bring all of the available data together for correlation of the material. A literature survey of existing knowledge on the survival of organisms under varying conditions gives an opportunity to study the various factors affecting survival and in understanding its role in the epidemiology of disease.

These reports will be published in a number of sections, of which this is the first: I. Air-Borne Organisms; II. Organisms in the Body and Body Materials; III. Organisms in Culture Media; IV. Organisms in Food; V. Organisms in Insects; VI. Effects of Pressure on Organisms; VII. Effects of Radiation on Organisms; VIII. Organisms in Soil; IX. Organisms on Surfaces; X. Organisms in Water; and XI. Summary.

Acknowledgment

These reports were made possible, to a great extent, through funds made available on contract from Camp Detrick, Frederick, Maryland, and through the interest of Drs. Charles Phillips and Saul Kaye.

The literature survey was carried out in the Department of Bacteriology and Parasitology of The University of Texas Medical Branch, Galveston, Texas, where the facilities of the Medical Library were utilized to a great extent. Some foreign and obscure journals were obtained by microfilm through the Army Medical Library, Washington, D. C.

Most of the work was done on a part-time basis by personnel of The University of Texas, who were responsible for the searching, reading, tabulating and writing-up of the material. These workers were: Jean Cobb, Louis DeVito, William Dillen, Beatrice D. Engley, Mary Faubion, Norman Halbrooks,

* From the University of Texas Medical Branch, Galveston. This study was carried out under Contract with Camp Detrick, Frederick, Maryland. Present address of author, University of Missouri, School of Medicine, Columbia, Missouri.

Meredith Holladay, Maxine Munn, Pat Riddle, Martha Jordan Scott, Elise R. Stein, Jacqueline Terrill, Elfe Thomas, Wilbur White.

The Survival of Organisms in Aerosols

One of the important methods of transmission of disease producing organisms is through the air—air-borne infections. An aerosol is a suspension of particles in air, in this particular instance a suspension of microorganisms in air. In nature, organisms may get into the air from the wind blowing over water and soil, raising microscopic water particles and dust particles into the air and suspending them there. Potential pathogens get into the air of human environment from sneezing, coughing, and expectorating as well as from clothes, bedding, sweeping of floors and other similar sources. Under such conditions these factors affect spread of infection (1) the organism virulence; (2) the host susceptibility; (3) effect of environment of organism preventing or aiding it in survival.

Aerosols of microorganisms may be set up experimentally by man in open or closed areas to study various factors affecting survival. These factors may be divided for convenience as to (1) environmental factors and (2) organism factors. These aerosols may be of organisms not usually considered as air-borne infection organisms as we usually consider them in nature.

Environmental Factors Affecting. There are many major and minor factors which may affect the ability of an organism to survive in an aerosol. The medium in which the organism is grown and the medium from which it is suspended for spraying may aid or adversely affect the organism. The growth medium may supply nutrients for production of active, well-developed cells. The suspending medium provides osmotic effect as well as protection against drying too rapidly or slowly, protection against radiation and other forces.

The temperature of the air may affect the survival of an organism in an aerosol to a certain extent. Usually at higher temperature lower recoveries may be expected if the relative humidity (RH) is constant.

The RH of the air is very important in the survival of organism. Various organisms may respond differently to high and low RH but usually at a 50 per cent RH greater destruction of the organisms result. At high or low humidities better recovery may occur. As noted, individual organism types may respond dif-

ferently to different humidity levels. Some organisms are killed if they dry too rapidly so low RH levels are detrimental. Others appear to give lower recoveries if sprayed into very high humidity air. From theoretical grounds this might be explained on the basis that particles sprayed into saturated air may act as nuclei of rain drops and precipitate out or may merely hit one another and form large particles which stick together and then precipitate out of suspension.

The amount of radiation present affects the rate of organism survival in an aerosol. Organisms are destroyed in sunlight and artificial sources of ultraviolet more readily than if present in an aerosol in semi-darkness or in absence of all light or artificial radiation. The sunlight accounts for a great amount of destruction of air organisms along with desiccation.

The method by which organisms are rendered air-borne is important in their survival as aerosols. In nature, organisms covered with a film of moisture or soil or oily film of dust will be protected against desiccation and radiation. In experimental aerosol production, the type of spray device is of importance. The rate of spray, amount of shearing force put on the cell and the size of particle developing will affect survival in the air. Some sprayers may destroy the cells by the force of spray; others form large particles which do not stay air-borne for long periods. For infecting animals, particles smaller than 10 micra in diameter must be formed and viable organisms must be present.

In nature the amount of air movement is important in keeping the organisms suspended. Also important is the resuspension of organisms as secondary aerosols by air and wind movement as well as by human and vehicle traffic.

The method of sampling aerosols is important in evaluating the results. If present as large particles then they will settle out rapidly as may be sampled on "drop-plates." Various types of samplers may collect particles within a certain range—some particles may be too big and others too small. This must be taken into consideration. As mentioned previously, particles above 10 micra in diameter are too large to be respired by animals so that aerosols of particles may be quite viable but too large to cause respiratory infection.

Organism Factors Affecting. Each genus and species of organism may have its own characteristics which may protect it from destruction when present as an air-borne organism. The indi-

vidual strain of organism sometimes has certain characteristics which allow it to remain viable more or less long than closely related strains and species. Some organisms may be protected by their presence as a spore or by their capsule or resistant cell wall. All of these characteristics resist the forces of nature—desiccation, radiation and temperature changes as well. The size and shape of the organism and rigidity of the cell wall aid an organism to resist the forces present in spraying procedures.

The age of the cells (in the growth curve) at the time of spraying determines to a certain extent their resistance to desiccation, radiation and other factors. The length of storage before spraying may be another factor. The numbers of organisms will also affect the length of survival of the aerosol.

Summary

**Bacillus* (Table A 1) *B. anthracis* spores resisted ozone for 4 hours and could withstand direct sunlight for days. *B. subtilis* resisted UV and remained viable in air for 5–7 days, experimentally. *B. anthracis* apparently survived 200° F. for 24 hours. *B. megatherium* was transported for many miles by storms.

**Brucella* (Table A 2) *Brucella melitensis* survived in dust for 30–44 days experimentally. In nature it survived equally long. High rainfall areas have Brucellosis—arid areas none. Sunlight quite effective in killing organisms.

**Clostridium* (Table A 3) Vegetative cells susceptible to oxygen.

**Corynebacterium* (Table A 3) The diphtheria organism survived in dust for 7–102 days or longer. Organisms found in air from diphtheria patients. It survived 48 hours in air.

**Lactobacillus* (Table A 3) The organisms (*L. acidophilus*) settle out of air rapidly (5–10 minutes). Radiation is not too effective against it.

**Neisseria* (Table A 3) The *N. meningitidis* organism can travel and infect in wards up to 5 feet in distance.

**Vibrio comma* (Cholera) (Table A 3) During the cold months the organism dries and dies. Experimentally dried in air it may survive up to 108 hours.

**Diplococcus pneumoniae* (Table A 4) Survives only 42 minutes in sunlight but 42 hours in dark. RH of 50 per cent is very lethal but not with NaCl removed The lethal effect is raised with larger particles and higher temperatures. Organisms in floor dust for over one month. Usual survival in air is short but many survive for 48 hours.

**Escherichia coli* (Table A 5) Recoveries up to 25 per cent may be obtained in air. Faintly resistant to ultra-violet especially with increased humidity. Cigarette smoke protects from chemicals in air. It may survive up to 4 years in dust.

**Microorganisms* (Table A 6) General factors affecting survival of organisms at altitudes are presented. Some organisms found up at 70 thousand feet. In experimental studies factors affecting survival of organisms in general are

presented (temperature, radiation, chemicals, air conditioning). Scattered reports are listed for *Erwinia, Hemophilus, Malleomyces, Micrococcus, Proteus, Pseudomonas* and *Sarcina.* Several reports are present for *Serratia* as follows: The organism survives as long as one day in air. One report indicates using nose as sampler. Nose and throat fluids are toxic to *Serratia.* Low humidity destroys organism rapidly. Reports on *Proteus* suggest survival of 2–12 days.

**Mycobacterium tuberculosis* (Table A 7) Organism survives 8–14 days in dust and 4–7 days in sputum droplets but 2 months in sputum. Ultraviolet and glycols tested against organism.

**Pasteurella* (Table A 8) The plague organism dies rapidly on drying but is infective by air route. The tularemia organism is inhaled from grain dust to infect.

**Protozoa* (Table A 9) *E. hystolytica* cysts did not survive when air dried.

**Phage* (Table A 9) Survives air drying and can be disseminated through air.

**Rickettsia* (Table A 9) Typhus may be transmitted through air. *C. burneti* is reported in goat barn. Rickettsiae grown in egg and sprayed survive 30 minutes in air.

**Salmonella* (Table A 10) Sprayed into air, the organisms survive 8–24 hours. Higher Rh and temperature lower recovery. Dust protects the cells and organism survives sun rays for 4–10 hours.

**Staphylococcus* (Table A 11) RH of 50 per cent is lethal. Survives 3 days in air. Ozone not very toxic. Survives in floor dust for several days. May be quite resistant to UV light.

**Streptococcus* (Table A 12) Killed by sunlight in 4 hours but lives in dark for at least 65 hours and perhaps up to 2 weeks. Good survival in high and low humidity, but not 50 per cent RH. Infective as small particles (2 micra). Particles near 12 micra diameter not as infective. Survives in air for 48 hours. Sunlight and UV are detrimental. Organisms from air may survive in blankets for 4 months. There may be 40 per 10 cubic feet of air in hospital wards or up to 378 per cubic foot. Glycols may reduce population 90 per cent.

**Viruses* (Table A 13) May be found at high altitudes.

Influenza—Survives in dust less than 3 weeks. 50 per cent RH more effective in destroying virus than higher RH. Some disagreement, however, exists over RH effect. Virus from air on blankets survives drying. It may survive many days in air but glycols and sunlight destroy it rapidly.

Vaccinia—Survives in air 8 hours, being more susceptible to destruction than Streptococci. Resists radiation like bacteria.

Foot and Mouth—Survives 1 week in outdoor air and in dust.

Smallpox—Seasonal incidence indicates low humidity favors the disease.

Infectious Jaundice—Virus carried in air by being dust-borne.

**Yeasts, Molds and Fungi* (Table A 14) Some of these species are found at 10 thousand to 36 thousand feet high. Some may travel 100 miles or 300–400 miles or even as far as 1200 miles to infect plants. Some species isolated in Antarctica. Resistance to UV is high. Certain human diseases are affected by low RH and wind to dry and carry spores (coccidiomycosis) while others need high RH and lower temperatures such as Sporotrichosis.

TABLE A1

The Survival of Bacillus Species in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL			
B. anthracis			
Spores, dist. water, 25° C. Direct sun, strong wind.	Inoc. 8,000 col., recov. 0, 2 h.	Kruse	1895
Ozone, 4 h., 37° C.	No effect	Ransome	1901
Sunlight with air	24 h.	Roux	1887
Sunlight without air	> 83 h.	Roux	1887
Steam under pressure, autoclave, 15 lbs.	30 min.	Smyth	1921
B. subtilis			
Spores, spray drying.	Show small mortality	Bullock	1947
Air inlet temp., 180° C.	Show small mortality	Bullock	1947
Air inlet temp., 75° C.	10% killed	Bullock	1947
Air inlet temp., 55° C., liquid suspension, 30 sec.	50% killed	Bullock	1947
Dried state, 70° C., 40 min.	50% killed	Bullock	1947
Dried state, 110° C., 1 h.	Complete destruction	Bullock	1947
Moist container, 7.2%, 90° C., 1 h.	Destroyed	Bullock	1947
Dust filters and precipitators effect against bacterial droplets when it is considered at the present no occupied space can be made bacteria free.		DallaValle	1944
Exposed to U.V.	Innumerable col., 1 min., 42, 3 min.	Hart	1939
Suspended in air	62,225 (±72%) ergs/cm^2 necessary for sterilization of air.	Sharp	1940
Broth sprayed in air	2.6 bacteria/ft.^{3} air after 5 d.	Wells	1934
Dist. water sprayed in air.	10 bacteria/10 ft.^{3} air after 7 d.	Wells	1934
Infection depends more on viability of organism than on settling rate.		Wells	1934
OUTDOOR			
B. anthracis			
Dry air, 200° F.	24 h.	Smyth	1921
B. megatherium			
Dust	Evidence of transport during storm	Soule	1934

TABLE A2

The Survival of Brucella Species in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL			
B. melitensis			
Dust contaminated with urine	30 d.	Chief	1944
Dust	44 d.	Kennedy	1905
Ozone 4 h., 22° C.	No effect	Ransome	1901
OUTDOOR			
B. melitensis			
Dry dust of Malta	20–28 d.	Horrocks	1906
Dry sterile dust	20 d.	Horrocks	1906
Damp sterile soil	72 d.	Horrocks	1906
Dry sterile manure	69 d.	Horrocks	1906
Moist sterile manure	7 d.	Horrocks	1906
Moist unsterile manure	20 d.	Horrocks	1906
B. spp.			
Incidence of infection high where total rainfall high and negligible in semi-arid areas. The sterilizing effect of continuous sunlight seems to be paramount in checking dissemination while humidity, rainfall, sunlessness and congestion of animals favors spread.		Polding	1950

TABLE A3

The Survival of Clostridium, Corynebacterium, Lacto-Bacillus, Neisseria and Vibrio Species in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL			
C. diphtheriae			
Ozone, 4 h., 37° C.	Growth resumed after 8 d. incub.	Ransome	1901
Air	2 d.	Wells	1936
C. xerose			
Maleic and phthalic anhydrides are more active than their corresponding acids. The effective vapor conc. is 25% saturation for chemical disinfection of air.		White	1944
Lactobacillus acidophilus			
Those which became suspended in the air from liquid media either by natural or artificial means settle out rapidly in 5–10 min.		DuBuy	1947
Bacterial population somewhat reduced by radiation but not consistently.		DuBuy	1948
Vibrio cholera			
Water spray, cocoon thread.	Recov. 0, 1½ h.	Kirstein	1900
Water spray, silk threads.	Recov. 0, 72–108 h.	Kirstein	1900
In Calcutta	Death rate inc. with temp	Ray	1950

TABLE A3—(Continued)

The Survival of Clostridium, Cornyebacterium, Lacto-Bacillus, Neisseria and Vibrio Species in Air

Factor(s)	Survival	Reference	
INDOOR			
Corynebacterium diphtheriae			
Floor dust, dark, in vitro	7–102 d.	Crosbie	1941
Floor dust sweepings	5 wks.	Crosbie	1941
In air after oiling floor	Untreated, there were 7 colonies	Crosbie	1941
During and after sweeping	7 col. Gravis	Crosbie	1941
During quiet period	8 col. Mitis	Crosbie	1941
During bed making	1 col. Gravis	Crosbie	1941
Diphtheria patients	Expulsed from resp. tract by 10 of 50 patients	Duguid	1946
Drying, R.T., 25 min.	Inoc. 204 col., recov. 0, 1 h		
Drying, R.T., 60 min.	Inoc. 13 col., recov. 0, 1 h.	Jochimsen	1928
Dust, dried, R.T.	175 d.	Ouchterlony	1949
Dust	Long periods	Pressman	1937
In talking and coughing, diphtheria patients frequently emit droplets containing viable bacilli		Teague	1913
Air	48 h.	Wells	1935
Floor dust (Gravis str.)	1 mo.	Wright	1941
Floor dust	2 mo.	Wright	1941
Neisseria meningitidis			
Can be carried at night from a carrier to his neighbor unless bed space is > 5 ft.		Eagleton	1919
OUTDOOR			
Clostridium welchii			
Only sporulating forms survive when influenced by oxygen under pressure or atmospheric air conditions.		Ernst	1900
Corynebacterium diphtheriae			
Air, drying	Surv. long time in dust	Ernst	1900
V. cholera			
Coldest month of year, dries out		Rogers	1944

TABLE A4

The Survival of Diplococcus Species in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL			
D. pneumoniae			
Daylight, in simulated room environment	42 min.	Buchbinder	1942
Dark, simulated room environment	12 h.	Buchbinder	1942
Spraying into atmosphere from large susp. of broth, saliva or 0.5% saline produced high mortality rate at 50% R.H. At R.H. above or below this the survival was prolonged.		Dunklin	1948

TABLE A4—(Continued)

The Survival of Diplococcus Species in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL, Continued			
When saline free liquid used, the sharp peak in death rate at intermediate R.H. disappeared. The lethal effect of intermediate R.H. on pneumo-cocci atomized from saliva containing susp. is increased when the particle size of the droplets is increased or when the temp. is raised. A narrow range of R.H. near 50% is rapidly lethal for organisms freshly sprayed in air.		Dunklin	1948
INDOOR			
D. pneumoniae			
Organisms susp. in fresh atomized saliva	Recov. 95.3 immed. after spraying		
	Recov. 329 after 75 min.	Robertson	1942
Outside body	Brief duration	Robertson	1947
22° C., R.H. 50–80%	Within 10 min. all the pneumo. had disappeared from air at 50% R.H.	Robertson	1948
Floor dust, Types I & II	> 1 mo.	Stillman	1917
Air	48 h.	Wells	1935

TABLE A5

The Survival of Escherichia Coli in the Air

Factor(s)	Survival	Reference	
EXPERIMENTAL			
Air aerosols, after atomization	25% viable	Ferry	1951
Exposed to ultraviolet	Innum. col. at 1, 3, 8 min.	Hart	1939
Ozone, 4 h.	60 sec.	Ransome	1901
Air	1 day and 8 hrs.	Wells	1936
Broth and air, atomized, dark room	120 min.	Wells	1935
Ultraviolet lamp off	30 min.		
Ultraviolet lamp on	12 min.		
Ultraviolet lamp covered	15 min.		
Increased humidity	Inc. resistance to germicidal energy	Luckiesh	1942
Susp. in air	24,800, plus or minus 5.4% erg/cm^2 necessary for sterilizing air.	Sharp	1940
With ultraviolet the bactericidal action is greatest at low humidity. In an atmosphere of 45% R.H. it is about 10 times as lethal as at 90% R.H.		Elford	1942
Cigarette smoke has no germicidal effect and nulls germicidal activity of 10% hexyl resorcinol in propylene glycol.		Twort	1940
OUTDOORS			
Humid	Max. growth when most humid	Kopeloff	1922
Dust	4 yrs.	Savage	1903

TABLE A6

The Survival of Microorganisms in Air

Factor(s)	Survival	Reference	
ALTITUDE			
Bacteria and mold spores.	Found at 70,000 ft.	Armstrong	1936
Nutrient agar, 22° C., pH 7.2, 5,700 ft. above surface	103 col. Numerous bact. carried along with dust into atmosphere are not killed by light, heat and dryness of desert air	Brown	1930
Higher bacterial counts in clouds.		Dillon	1929
Lower count bacteria in clouds due to loss of electro-static charge that caused dislodging of particles which adhered to plane		Durham	1941
Bacteria, moist film, ozone	More easily killed than when in dry state.	Elford	1942
Bacteria sprayed into air, R.H. 66%, ozone, .15 ppm., 21° C., 15 min.	> 99% killed	Elford	1942
Ozone conc. in excess of 1 ppm. in an atmosphere of 60–80% R.H. required to produce good sterilization.		Elford	1942
Ten times as many germs in air on clear days as on foggy days		LeGuyon	1931
Subnormal sunshine and precipitation deemed responsible for survival of infectious agents in air. High cooling power due to strong winds reduced resistance of people		Meissner	1940
Decreasing number of bacteria proceeding from basement to 4th floor		Parvis	1948
Chance association with dust particles increases survival of air-borne organisms		Personnel of Navy Res.	1943
4 cases described bitten by insect native to S. America in Mojave desert		Schlotthauer	1940
Study of number of organisms found in upper atmosphere ranging from 28 to 280		Timmon	1949
Agar plates at 19,000 to 28,000 ft. produced no organisms		Walker	1935
EXPERIMENTAL			
Rats, G.P. and rabbits exposed to aerosol of bact. spores		Ames	1949

TABLE A6—(Continued)

The Survival of Microorganisms in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL, Continued			
Propylene glycol less efficient than phenol when applied as a paint and better than phenol when evap.	Certain areas of large spore conc. The greater the volume of tidal air, the greater the number of organisms in the lungs	Baker	1944
A number of air-borne bacteria were able to survive for a given time but decreased by raising R.H. from 40 to 60% or higher		Baker	1941
Sterilizing of air by U.V. radiation effective when the organisms are dispersed in droplet nuclei, the smallest particles being most vulnerable		Bourdillon	1948
Air from sneezing	100,000 bacteria remain in air over 1 min. 16,000 still in air over 30 min.	Bourdillon	1942
Air with hypochlorite spray, 65° F., R.H. 66%	All or most of bacteria emitted can be killed in 3-4 min. by spray of NaHC10 in conc. of 2.1 cc./1,000 cu. ft. air.	Bourdillon	1942
	Lower limit of effective humidity is below 60% at 70° F.	Bourdillon	1942
Apparatus for determination of penetration of particulate air-borne material through nose described. Particles greater than 5 micra are filtered out.		Boyland	1947
In general, moving hot air is more effective in sterilizing of plane polished surface than still hot air of same temp.		Breinl	1935
Size of aggregates of micellae increased with concentration of saline, with increase time in sedimentation chamber.		Dautrebande	1948
Fan distribution of droplet nuclei. Disappearance of 90% in 30–60 min. Nuclei greater than 8 micra in diameter survived 20 min. Nuclei greater than 4 micra survived 90 min. Smaller nuclei survived 30 h.		Duguid	1946
Bacteria carried in handkerchief after 2 days use liberated on mild manual shaking.	Recov. 14,720	Dumbell	1948
Organisms heated in presence of steam are maintained in state of intermediate hydration which makes them more susceptible to killing action of high temps. than would be the case if allowed to dry out completely.		Dunklin	1948
50% solution calcium chloride spread on floor will prevent dust 4–6 weeks.		Galambos	1942
Technic outlined for determining particle size distribution of viable air-borne bacteria.		Goldberg	1950
Means of sampling bacterial aerosols		Goldberg	1951
Wave length of 2537 Å highly efficient in inactivating many disease agents.		Hollaender	1946
Factors which influence efficacy of glycol vapor: 1) RH. 30–50%. 2) Greater below 72° F. than above. 3) More effective with dirt and dust than alone.		Hamburger	1945

TABLE A6—(Continued)

The Survival of Microorganisms in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL, Continued			
The physiological and biological concepts for bactericidal irradiation of air have been fairly well-established by various investigators.		Hart	1944
Relatively low intensity U.V. of 2537 Å will interfere with cellular division and readily inactivate many disease agents which are not protected by other substances.		Hollaender	1942
House dust, 30–35° C., R.H. 53–63%	Recov. 225/10 cu. ft. after 30 min.	Hollaender	1944
The bactericidal effect of glycols varies markedly with changes in temperature and in humidity, decreasing rapidly with temperature rise and reaching maximum between 40 and 60% R.H.		Krueger	1944
Bacteria, oxygen, exposed to sunlight	3 h.	Kruse	1895
Bacteria, hydrogen, exposed to sunlight	>7 h.	Kruse	1895
Indicates that inhalation of dust borne pathogens may be important mode of spread within group.		Lemon	1948
Construction of apparatus for experimental study of respiratory infections.		Leif	1950
At a constant saturation level of glycol vapor, there was found to be approximately a two-fold increase in the rate of bactericidal action with each 15° F. increase in temp.		Lester	1950
At high humidities the particles in equilibrium contain so much water that highly bactericidal concentration of glycol are unattainable. A limit of 290° F. should be employed in vaporizing.		Lester	1950
Equation given for determining effect of radiation on bacterial population.		Lidwell	1948
Air suspension, Low R.H. Bactericidal effect.		Lidwell	1948
The falling off in effectiveness at lower humidities of all bactericides considered appears to be a function of the bacteria carrying particles themselves. The maximum killing rate is attained only at vapor concentration near or in excess of sat.		Lidwell	1948
Vapors of most common aerial disinfectants disappears from the air at an appreciable rate by one or more of a number of processes which include aerial oxidation, condensation upon surfaces, adsorption by surfaces. The disappearance of the vapors from the air approximated a logarythmic law over a range of concentrations.		Lidwell	1948
Lactic acid is cheap bactericide. Reached peak between 60–85% R.H.		Lidwell	1948
Bactericidal illumination is much slower with dust than with culture spread on slides or petri dishes at room R.H. (60%). Low intensity U.V. and fluorescent lighting of good intensity appeared to destroy the various organisms about five times as fast as natural death rate in dark.		Lidwell	1950
The treatment of cotton and woolen bed clothes and other fabrics with oil emulsion is not readily affected by changes in R.H., temperature or small variation in concentration of oil emulsion used.		Loosli	1946
Aliphatic alpha hydroxy carboxylic acids are good bactericidal agents for air disinfection.		Lovelock	1948

TABLE A6—(Continued)

The Survival of Microorganisms in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL, Continued			
One gram of propylene glycol aerosol in 2,000,000 of air effected complete sterilization of an atmosphere containing as many as 500,000 bact./l. of air.		Miller	1942
Filters prepared from gelatin permit the most successful total recovery by culture procedures from an inoculum of a known number of organisms in contaminated dust.		Mitchell	1949
Air, U.V. 20 micro watts per sq. cm. (2,537 Å)	Adequate disinfection 250–500 sec.	Mudd	1944
U.V. 30 micro watts per sq. cm. (2,537 Å)	167–334 sec.	Mudd	1944
Review of methods used in preparing aerosols		Muller	1940
The two primary conditions for the successful application of U.V. for disinfection of air are: 1) the supplementary irradiation with dust suppression measures since U.V. light is not efficient against bacteria protected by dust. 2) attain a radiation of sufficient intensity but not so high as to affect the eyes or skin.		National Res. Council	1947
Glycols are most effective at R.H. between 40–60% and temp. below 80° F.		Niles	1946
Effectiveness of any compound as an aerial germicide depends upon the extent of condensation of its vapor on air suspended bacteria and on the rate at which the resulting conc. of germicide can produce death of the organism. At any R.H. the killing action is greater the more closely the germicide vapor concentration approaches the saturation point.		Puck	1947
Glycol vapor conc. maintained at a level just below the fogging point were found to be as effective as supersaturated atmosphere. Presence of visible mist of glycol in no way harmful.		Puck	1945
Air, 80° F. and below, R.H. 45–70%, sprayed with propylene glycol	Maximum bactericidal action	Puck	1943
Ozone shows no remarkable effects on pathogenic bacteria		Ransome	1901
No difference of U.V. bactericidal action over a R.H. range of 35–95%.		Rentschler	1940
Temperature of organisms (within viability range) has no effect on action of U.V.		Rentschler	1941
Airborne bacteria about 1/10 as resistant to U.V. radiation as when in liquid suspension or on agar.			
More resistant at high R.H. than in low R.H.. Lethal radiation less if bacteria first exposed to heat.		Rentschler	1941
Short intense dosage of bacteria with U.V. is less lethal to E. coli than long mild exposures.		Rentschler	1939
Treatment of bedclothes with medicinal paraffin reduces amount of dust that can be liberated from blankets by 90%.		Van den Ende	1941
Epidemic spread of contagion depends upon deficiency of air supplied per susceptible person. The ten-fold increase in winter ventilation or its equivalent in U.V. radiation does control epidemic spread.		Wells	1943
Methods of standard bacteriologic procedure.		Wells	1946
Examination at autopsy failed to reveal any difference between animals kept in glycolized air and those in ordinary room atmosphere.		Wise	1947

TABLE A6—(Continued)

The Survival of Microorganisms in Air

Factor(s)	Survival	Reference	
INDOOR			
Triethylene glycol acts as dehumidifying and germicidal agent. Introduced as vapor shows 90% reduction of bacteria in air.		Barrett	1947
Definite decrease in incidence in severity of infections of the upper part of respiratory tract noted among children kept in irradiated ward as compared with control.		Bayenberg	1940
Irradiated materials had much greater bactericidal action than non-irradiated material.		Beckhold	1937
Number of colonies diminished proportional to care taken in use of caps and mask.		Brown	1916
90–95% fewer organisms obtained from oiled blankets than from control blankets.		Dingle	1946
Gram negative bacteria in air. R.T., Combined spectrum killed most bacteria in 15–30 sec. of U.V. radiation. Preceding freezing with liquid air makes gram negative bacteria more strongly suscept. to U.V. than gram positive spore formers.		Gartner	1947
Analysis of bacteria found in large rural central schools. 207 Strep. isolated from rooms irradiated with U.V., compared to 318 from non-irradiated rooms.		Gilcreas	1950
Marked decrease observed in total rate of incidence of upper respiratory infections following use of propylene glycol vapors.		Harris	1945
Gives various organisms detected in operating room.		Hart	1938
Bacteria have definite variations in rate of multiplication and amount of growth with changing conditions of moisture.		Kopeloff	1922
Filters of air conditioner more effective when partially clogged with dirt. Neither furnace heat nor centrifuge action of blower appears to have any influence on number of organisms recirculated.		Lemon	1944
Fluorescein dispersed into air of room as powder. Recov. from dust after 20 sweepings.		Lowbury	1950
Saliva in air	36 h.	Luckiesh	1947
Organisms sprayed in air	3000 org./cu. ft.air. 14% of initial value, 2 h.	Luckiesh	1947
37° C., textile mill	Colonies grew more than at R.T.	Matuso	1943
Bacteria counts higher in Jan., March, April, Dec. Lowest in Aug. and Sept.		Matuso	1943
If the entrance to lab. is hung with cloth soaked in carbolic acid and the window locked tightly there is considerable reduction in bacterial content of air in the room.		Oesterle	1938
Bacterial content of morning air in operating room twice that of afternoon. Count is lower in the summer.		Rice	1941
Use of U.V. lamps in ducts of air conditioner significantly reduced bacterial count.		Roberston	1940
Room with oiled floors had 70% reduction in air-borne bacteria during maximum activity.		Robertson	1944
U.V. lamps effective in reducing bacteria and preventing spread in air ducts.		Roberston	1939
R.T., R.H., 15–40%, glycol saturation 40–100%	2–3 min.	Robertson	1949

TABLE A6—(Continued)

The Survival of Microorganisms in Air

Factor(s)	Survival	Reference	
INDOOR, Continued			
The higher the concentration of glycol the more rapid the the bactericidal action. Concentrations of 70–80% produced rapid killing.		Robertson	1948
Air in nursery. No nurse in cubicle for 1 h.	9 col./10 cu. ft. air	Rosenstern	1948
Nurse in cubicle for 10 min. for diapering infant.	299 col./10 cu. ft. air	Rosenstern	1948
No air conditioning, count aloud from 1–50 without face mask	25 col./10 cu. ft. air	Rosenstern	1948
Air conditioning alone did not prevent spread of respiratory cross infection. Germicidal irradiation of upper air together with air conditioning reduced number of respiratory cross infections. Flannel gauze mask worn by attendants of value in preventing cross infection.		Rosenstern	1948
Prevention of cross infection by U.V. and air conditioning.		Sauer	1942
Tabulation of 1,342 infections acquired in the laboratory. Recognized accidents in 215 instances 308 cases research, 455 cases diagnostic work, 25 cases product of biologicals, 29 cases class.		Sulkin	1951
Air before sweeping	0.7/cu. ft.	Thomas	1941
Air during sweeping	24.1/cu. ft.	Thomas	1941
Air after sweeping	30 min.	Thomas	1941
Treatment of bed clothes with liquid paraffin causes 95% reduction in number organisms distributed in air during bedmaking.		Thomas	1941
Samples of air from U.V. hospital ward averaged 5 organisms /cu. ft.		Wells	1940
U.V. irradiation checked epidemic spread of contagious diseases during cold weather, when air dry. Not effective during moist weather.		Wells	1943
Bacterial reductions of 20–25% with use of U.V. irradiation in barracks.		Wilimon	1948
Rate of disappearance of bacteria from air follows logarithmic relation with respect to time. Disappearance rate increases as R.H. increases. Temp. did not effect rate of disappearance. NaOCl aerosol can effect reduction in the bacteria in the air but requires a high mist conc. The R.H. of air influences effectiveness of aerial bactericides. The vapor pressure of the bactericides influences its use and effectiveness as aerosol. Low R.H. more favorable than high for viability of air suspended organisms.		Williamson	1942
Indoor dust	3–5 million bact./gm.	Winslow	1912
OUTDOOR			
Strong proteolytic protein, 20° C., Incub. 7 d.	Aeration stimulates bact. growth, decomposes casein, produces ammonia, decreases nitrogen.	Allen	1940
10 to 11 mg/cu. meter of absolute humidity or greater allows organisms to survive long enough for infect.		Bey	1948
Air of sewers does not give off germs.		Jacobi	1894
Organisms in air, heavy rain. 80% reduct., 4 h.		Lewis	1907

TABLE A6—(Continued)

The Survival of Microorganisms in Air

Factor(s)	Survival	Reference	
OUTDOOR, Continued			
Organisms in air, light rain.	30% reduct., 4 h.	Lewis	1907
Organisms in air, R.H. under 70%	214/sq. ft.	Lewis	1907
Organisms in air, R.H. between 70 and 80%	144/sq. ft.	Lewis	1907
Air windward of town	11/sq. ft./min.	Lewis	1907
Air leeward of town	27/sq. ft./min.	Lewis	1907
R.T., textile mill	Colonies from external air thrive more than at 37° C.	Matuso	1943
Effectiveness of U.V. light decreases rapidly with increasing R.H. above 55 or 60%. The rays are more efficient against small particles than large.		Perkins	1947
Samples of outdoor country air average 14.5 cu. ft. air/lactose fermenting organism. Samples of outdoor city air average 10.2/cu. ft.		Wells	1940
Street dust	49,200,000 bact./gm.	Winslow	1912
Air-borne marine bacteria very resistant but killed by U.V. radiation just as readily as fresh water or terrestial species.		Zobell	1942
Marine organisms collected on towers 30 miles inland. Terrestrial organisms collected 130 miles at sea. Collections were proportional to wind velocity.		Zobell	1936
ALTITUDE			
Can be transported almost limitless distances horizontally depending on ability to survive atmospheric environment.		Jacobs	1940
Bacteria found above 19,000 ft. at —26° C., survived >48m.		Proctor	1934
Numerous bacilli, staphylococci and micrococci found above 20 thousand feet.		Proctor	1935
Windy weather prior to flights resulted in increased counts.		Proctor	1942
Flights 50–60,000 meters. Bacterial distribution was Coccaceae 50.3%, Bacteriaceae 3.65%, Bacillaceae 39.8%, Actinomycetaceae 3.10%, Spirillaceae 0.15%.		Skrsynska	1949
EXPERIMENTAL			
Erwinia amylovora			
R.H. close to 0.	Viable and infectious after 1 yr.	Rosen	1936
Hemophilus pertussis			
Air	1 h.	Wells	1936
Malleomyces mallei			
Ozone 4 h., 37° C.	Growth resumed after 8 d. incubation	Ransome	1901
Micrococcus candidus			
Aerosols, air, after atomization	Resistant to shock. Same vitality in aerosol as in suspension	Ferry	1951
Proteus vulgaris			
Exposed to U.V.	61 col. 1 min., 2 col. 3 min.	Hart	1939
Pseudomonas pyocyaneus			
Exposed to U.V.	Innum. col. 1 min.; 38 col. 3 min.	Hart	1939
Ozone 4 h.	60 sec.	Ransome	1901

TABLE A6—(Continued)
The Survival of Microorganisms in Air (Addendum)

Factor(s)	Survival	Reference	
EXPERIMENTAL, Continued			
Pseudomonas aeruginosa			
Suspension in air	16,000 (±4.7%) ergs/cm² necessary for sterilizing air	Sharp	1940
Sprayed into air	<1 d.	Wells	1934
Air	<1 d.	Wells	1936
Sarcina lutea			
Swabbed on tongue, nasal mucosa and tonsil crypts	Usually impossible to recover	Bloomfield	1919
Sarcina spp.			
Air, R.T., R.H. 70–72%, Triethylene glycol vaporized with hot air	82% killed, 24 h.	Gruen	1949
Serratia marcescens			
Suspension in spray reduced to dust, R.T.	Disappeared at rate of 1.5–0.17 mm/sed. Remained floating in quiet air of room 4 h.	Flugge	1897
Water spray	Recov. 0, 27–33 h.	Kirstein	1900
Serratia marcescens			
Ozone, 4 h.	60 sec.	Ransome	1901
Air breathed in thru nose and mouth	Recov. 91%, 10 min. after spraying bacteria in air		
Size of particles important:			
Large particle	87% during spraying		
Med. particle	85% 6 sec. after spray	Rooks	1939
Small particle	62%, 10 min. after spray		
Greater recoverability of a dynamic cloud in wet than in dry atmosphere		Rosebury	1947
U.V. light	15 min.	Rosenstern	1942
Suspension in air	20,700 (±4.7%) ergs/cm² necessary for sterilizing air	Sharp	1940
Air, 29° C., sun shining	3 min. after spraying, 6,000 col.	Teague	1912
	30 min. after spraying, 1 col.	Teague	1912
Pure cult. in nasal passage	Shows nearly all organisms destroyed before reaching nasopharynx	Thomson	1896
Sprayed into air	<1 d.	Wells	1934
Air	<1 d.	Wells	1936
Form and structure of organisms may be factors affecting persistence and viability and this may be related to infectivity.		Ferry	1951
Wave length 25 micra acts stronger against Gram neg. organisms. Chromogenic bacteria showed conspicuous stimulation at 313 micra. Spores were killed by U.V. just as fast as vegetative forms.		Gartner	1947
The rate of kill of air dried bacteria was highest at low humidity (15–20%); with rising R.H. the death rate decreased. As air-drying period increased, the rate of kill by glycol diminished. Glycol vapor was effective against particles as large as 8–10 micra in diameter but not killed as rapidly as those of 5–4 micra or less.		Robertson	1951

TABLE A6—(Continued)

The Survival of Microorganisms in Air (Addendum)

Factor(s)	Survival	Reference	
INDOOR			
Proteus morgani			
Dust of ward	2–12 d.	Hoare	1943
Bacteria counts in test barracks lower than control following U.V. radiation. At the end of the study open plates were found to be a poor method of air sampling because stray irradiation at 5 ft. level induced a 19% reduction in counts.		Jarrett	1948
Respiratory microbes, air U.V., 2537 Å.	Recov. 0.5%, 2 h.	Knowles	1950
Concentration of air-borne bacteria in army barracks depended on (1) number of men present at one time (2) amount of and type of activity (3) largest number bacteria found during greatest activity.		Lemon	1944
Air-borne bacteria in major surgery roughly proportional to number of people present in surgery and to activity on floor proper of surgery.		Nisbet	1938
Microbes in air	Longer in humid than in dry air	Rochaix	1931
Air, ward, during bed making	Quiet, 22/cu. ft. air Beds in making, 510/cu. ft. air	Rountree	1946
OUTDOOR			
Hemophilus influenzae			
Death rate high during 4 mo. period of high R.H.		Barreto	1948

TABLE A7

The Survival of Mycobacterium Tuberculosis in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL			
Water droplets carry microbes. G.P. not infected after inhaling dry tubercle bacilli for 1 h., but infected in few seconds inhaling humid ones. Rain does not play important pathogenic role. Fog does play pathogenic role.		Aimes	1933
Droplets on plate, 45° C., 20–38 cm. from speaking or coughing persons mouth.	300–600 times increase in count	Flugge	1921
Loud speaking	40–1,000 times increase in 30 min.	Flugge	1921
Drops from bronchial tree in 20 coughs.	100–8,000 times increase	Flugge	1921
Sputum of tuberculous patient	50,000 bacilli/mg.	Flugge	1921
Tubercle bacilli can be recoverd from macroscopically normal lung tissue of rabbits several weeks after primary infection.		Heppleston	1949
When radiant energy of low intensity, it reduces the incidence of T.B.		Lurie	1944
U.V. of 2537 Angstroms exercised protective influence against natural air-borne contagion.		Lurie	1944
Ozone, 37° C., 4 h.	No effect	Ransome	1901
Use of saline solution in the Wells centrifuge will not permit collection of tubercle bacilli in atmosphere. Because of size, the bacilli fall out of atmosphere rapidly. When expelled from mouth they are enclosed in albuminous material which tends to stick to whatever it contacts.		Sim	1939

TABLE A7—(Continued)

The Survival of Mycobacterium Tuberculosis in Air

Factor(s)	Survival	Reference	
INDOOR			
Dust in air of rooms of tuberculous persons, R.T.	1 of 50 G.P. inoc. with dust died with T.B.	Augustine	1929
Surface dust from rooms of tuburculous families, R.T.	Found in dust six of 24 observations	Augustine	1929
Dust from clothing of tuberculous families, R.T.	10 of 62 inoc. G.P. died of T.B.	Augustine	1929
Washings from childrens hands, R.T.	No results	Augustine	1929
Open pulmonary tuberculous patient	Expulsed from resp. tract by 10 out of 20 patients	Duguid	1946
0.5–0.1 mg. cult./cc. water sprayed in G.P. vicinity	Bacilli in bronchi 1–72 h. Negative 144 h.	Heymann	1908
Dust	8–14 d.	Kirstein	1905
Sputum droplets	4–7 d.	Kirstein	1905
Hypochlorite and quaternary ammonium compounds	Weak tuberculocidal effect	Klarmann	1951
More emphasis than usual should be laid upon measures to prevent contamination of air. Specific experimentation has proved that U.V. has marked favorable effect.		Long	1951
The use of glycols as vapors is suggested for killing tubercle bacilli in closed spaces.		Potter	1944
Dust	Long periods	Pressman	1937
Aspiration of 1.4 cu. ft. air/min. not efficient for air sampling for isolation of T.B.		Pressman	1937
Sputum in air shaft	45 d.	Ransom	1905
Dust of T.B. wards	Can withstand drying	Rogers	1920
Sputum of floor	2–2½ mos.	Sawizky	1891
Dust, room, hospital and home	Long periods	Thomas	1941
OUTDOOR			
Dust, sunlight, dry	72 h.	Caldwell	1925
Mixed with sterile dust, direct sun rays	5 h.	Sweany	1919
Inhalation of a few tubercle bacilli in the nuclei of droplets coughed or sneezed into atmosphere is of greater consequence than from larger number of organisms in coarse particles which are strained out in the upper resp. passage and ingested.		Wells	1948

TABLE A8

The Survival of Pasteurella Species in the Air

Factor(s)	Survival	Reference	
GENERAL			
P. pestis			
Dust	Dries rapidly	Eskey	1938
P. tularense			
Streams and grain in Russia	Rats and mice are vectors	Maximow	1947
Tularemia infection acquired by inhalation of dust from threshing mills contaminated by infected rodents.		Ayres	1948

TABLE A9

The Survival of Protozoa, Metazoa, Bacteriophage and Rickettsiae Species in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL			
Bacteriophage			
Transmitted through air with droplets and dust in manner entirely analogous to transmission of bacteria.		Colvin	1932
Colloids protect bacteriophage against drying both in vacuo or in air.		Kriss	1948
Rickettsiae spp.			
Dyer Nine Mile, Henzerling str., saline susp. yolk sac.	30 min.	Ransom	1951
INDOOR			
Rickettsiae, typhus			
May be transmitted by air		Loffler	1942
OUTDOOR			
Entamoeba histolytica, cystic			
Air dried	Recov. 0	Kuenen	1913
C. burneti			
Air of goatery	Known infection in herd contaminates air considerably	Lennette	1951

TABLE A10

The Survival of Salmonella Species in the Air

Factor(s)	Survival	Reference	
EXPERIMENTAL			
S. typhosa			
Paper slips in vapor of 35 gms. phenol/1000 cu. ft.	Recov. 0, 1 hr.	DeOme	1944
Water spray	Recov. 0, 27–30 hrs.	Kirstein	1900
Ozone, 4 hrs.	60 seconds	Ransome	1901
Ozone, 4 hrs., 22° C.	No effect		
Air	8–24 hrs.	Wells	1936
S. dysenteriae (Hiss Y)			
Air	8–24 hrs.	Wells	1936
S. paratyphosa			
Air	8–24 hrs.	Wells	1936
S. pullorum			
Increasing RH from 15–80% increases death rate. Given RH with increase from 28–37° C. death rate increased. The combined effect of increased temp. and RH appears to be cumulative.		DeOme	1944
INDOOR			
S. pullorum			
Air, 28° C., RH 15%	50% death, 27.5 sec.	DeOme	1944
Air, 28° C., RH 46%	50% death, approx. 7 sec.		
Air, 37° C., RH 42%	50% death, 5 sec.		
When dispersed from broth into dust-free air its death rate was greatly decreased indicating that certain materials dispersed with bacteria may have a marked protective action.		DeOme	1944
OUTDOOR			
S. typhosa			
Direct rays of sun	4–10 hrs.	Osler	1901
S. enteriditis			
Open air, broth plate	Recov. on 5 occasions	Hewlett	1905

TABLE A11

The Survival of Staphylococcus Species in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL			
S. albus			
0.2 mg/1. of propylene glycol, R.H. 41%:			
Immed. after spraying	Control, 9750 col., 1 cu. ft. test	Bigg	1944
15 min. after spraying	Control, 9360, 1 cu. ft.		
30 min. after spraying	Control, 9260, 1 cu. ft.		
60 min. after spraying	Control, 3220, 1 cu. ft.		
Sprayed into air, R.H. 50%	Lethal for organisms		
R.H. on either side of 50%	Prolongs survival	Dunklin	1948
Sprayed in aerosol. Safe ozone conc. (0.04 ppm.) in R.H. 160–90% exerted disinfectant action.		Elford	1942
Exposed to U.V.	66 col.1 min., 2 col. 3 min.	Hart	1939
U.V. Light	26,200 ergs/sq.cm. sufficient to kill all suspended bacteria present with exposure of 1.06 sec.	Sharp	1938
Suspended in air	23,300 (±5.1%) ergs/cm² necessary for steril. air	Sharp	1940
S. aureus			
Dried in nasal secretion in handkerchief	Lg. number surv. >1 mo.	Duguid	1948
Suspended in air	26,500 (±5.7%) ergs/cm² necessary for steril. air	Sharp	1940
Sprayed into air	3 d.	Wells	1934
S. citreus			
Exposed to U.V.	102 col. 1 min., 6 col. 3 min.	Hart	1939
S. pyogenes			
Greater numbers released in dust than by sneezing. 50% reduction when wearing surgical gown.		Duguid	1948
S. spp.			
Killed rapidly at 70–90% R.H. with sprayed HC10. Bactericidal effect reduced in atmosphere of extremely low carbon dioxide content (0.001%) compared with behavior in N_2. Organisms in finely dispersed mixture killed more rapidly than those in heterogenously sprayed particles.		Elford	1945
Wounds cross infected	18 of 28 surgical wounds	Rountree	1947
INDOOR			
S. albus			
Air during and after cleaning	Found in air during 1st and 2nd hrs.	Furtherer	1946
Dried on glass, 15–16° C., R.H. 15–60%, Exp. to ozone for 5, 6 and 9 hrs.	No killing or inhibition of bacteria	Galli-valerio	1914

TABLE A11—(Continued)

The Survival of Staphylococcus Species in Air

Factor(s)	Survival	Reference	
INDOOR			
S. aureus			
Air during and after cleaning	Found in air during 1st and 2nd hrs.	Furtherer	1946
Most of infections from air contaminated by operating room personnel and patient		Hart	1937
Floor dust	Several days	Lidwell	1950
Survives better at low than at high R.H.		Lidwell	1950
Air of operating room	Survived in U.V. light	Phelps	1939
S. spp.			
R.H. 5–80%	Maximum humidity effect between 60 and 70%	Robertson	1948
OUTDOOR			
S. aureus			
Conc. calcium chloride, R.H. 35–48%	Least growth with minimum humidity	Kopeloff	1922

TABLE A12

The Survival of Streptococcus Species in the Air

Factor(s)	Survival	Reference	
ALTITUDE			
S. spp.			
Sprayed with 5 ml. of 1% hypochlorite solution	95% killed; 5% recov.	Andrews	1940
Oiled blanket, plates exposed 5 min. duringand after beating	170 colonies	Andrews	1940
Unoiled blanket, plates exposed 5 min. during and after beating	1,030 colonies	Andrews	1940
EXPERIMENTAL			
S. pyogenes (Group A)			
Exposed to daylight in simulated room envirn.	252 min.	Buchbinder	1942
Dark, simulated room envirn.	65 hrs.	Buchbinder	1942
Sprayed in air, settle on filter paper, daylight	6 hrs.	Buchbinder	1941
S. pyogenes (Group B)			
Exposed to daylight in simulated room envirn.	66 min.	Buchbinder	1942
Dark, simulated room envirn.	132 hrs.	Buchbinder	1941
Sheep blood agar, 18 hrs., 37° C., dark room, susp. in air	Remain viable in dust of room at least 2 wks.: some impairment of virulence		
Exposed to ultra violet:		Hart	1939
1 min.	98 colonies		
3 min.	12 colonies		
Air and triethylene glycol, CO_2, 70° F., R.H. 40%	10–15 min.	Wise	1949

TABLE A12—(Continued)

The Survival of Streptococcus Species in the Air

Factor(s)	Survival	Reference	
EXPERIMENTAL, Continued			
Bactericidal effect of irradiation of beta strep. appeared to depend on both R.H. and the susp'n med. Organisms in films prepared from serum broth cults containing dust behaved like those of dust and were more resistant under dry conditions. Org. in films from extracts of dust or from serum broth culture were more sensitive under dry conditions.			
S. pyogenes (Group C)			
Sprayed into air, R.H. 50%	Rapidly lethal	Dunklin	1948
Sprayed into air, R.H. above or below 50%	Increases survival	Dunklin	1948
Propylene glycol vapor conc. 0.2 mg/1, R.H. 42%, 1 cu. ft. immediately after spraying:	4,166 colonies	Bigg	1944
15 min. after spraying	3,120 colonies	Bigg	1944
30 min. after spraying	1,760 colonies	Bigg	1944
60 min. after spraying	442 colonies	Bigg	1944
Sprayed in aerosol. Ozone in safely tolerated conc. (0.04 ppm) in R.H. of 160–90% exerted a disinfectant action.		Elford	1942
S. pyogenes (Group C)			
Infections mainly due to particles smaller than 1.8u in diameter. As bacteria median diameter increases from 1–12u the aerosol dose had to be increased 10,000 times to kill 50% of animals. Pulmonary infection occurred much more readily than URI.		Sonkin	1951
S. pyogenes			
Sprayed into air	Rapidly killed in dry atmos., but protected in moist.	Wells	1942
R.H. 40–70%	Disinfection most apparent here	Wells	1942
Sprayed into air	Many float for many hrs. after all droplets have evaporated.	Buchbinder	1938
Exposed to room envirn.	No evidence that property of any strains were adversely affected	Buchbinder	1941
Oiling blankets, bed linen, garments, and floors reduced bacteria and hemolytic strept. of air during bed making to 91–98% below control ward.		Cruickshank	1947
S. viridans			
Exposed to daylight in simulated room envirn.	44 min.	Buchbinder	1942
Dark, simulated room envirn.	26 hrs.		
Sprayed in air, settled on filter paper, sunlight	50%, 5 min.	Buchbinder	1941
In dark and sunlight	Survival ranged from 14–93%	Buchbinder	1941
Wounds	3 of 82 surgical wounds	Rountree	1947
S. salivarius			
Sprayed in aerosol. Ozone in safely tolerated conc. (0.04 ppm.) in R.H. of 160–90% exerted a disinfectant reaction.		Elford	1942

TABLE A12—(Continued)

The Survival of Streptococcus Species in the Air

Factor(s)	Survival	Reference	
EXPERIMENTAL, Continued			
S. zooepidemicus			
Atomization reduced chain length by 50%. Recov. of viable organisms decreased with distance from atomizing nozzles when conc. cell susp'n used no. of mo. required to infect was larger by air-borne route than by direct nasal route.		Shechmeister	1950
S. spp.			
70–90% R.H., HC10 spray	Killed rapidly	Elford	1945
Irradiation classrooms	207 colonies	Gilcreas	1950
Non-irradiation of classrooms	318 colonies	Gilcreas	1950
Strep. susp'n in air remain viable at least 2 wks. in dust of room with some impairment of virulence.		Buchbinder	1941
INDOORS			
S. pyogenes			
Blankets, ENT ward	14,400–7,344,000/cu. ft. of air	Rountree	1946
Use of oil, water, and Roccal emulsion	Reduced no. in air 33–63%	Shechmeister	1947
Air	48 hrs.	Wells	1935
Dust	25 days	White	1936
Floor dust	67% of 185 samples	Williams	1949
Air from patient:			
with cellulitis, PM.	3 recovered	Willits	1941
with cellulitis, AM.	40 recovered		
while making bed	6 recovered		
while making bed briskly	14 recovered		
Room air, scarlet fever ward, blood agar plates, exposed to air 3 hrs.			
AM.	334 colonies	Allison	1937
Afternoon	228 colonies		
PM.	19 colonies		
Air in infant ward	Total counts for air in UV wards consistently lower than control ward	Brooks	1942
Air in scarlet fever ward	Absent at night, rise in AM, slowly falls in PM	Brown	1937
Found in congregation in cities		Buchbinder	1938
Dust	Several weeks	Cruickshank	1938
Oiled beds	26 of 307 cult. positive	Dingle	1946
Unoiled beds	160 of 441 cult. positive		
Droplets from cough	39 of 87 patients were throat carriers	Duguid	1946
Hospital dust, air settled on filter paper in Petri dish, dark	65 hrs.	Garrod	1944
sunlight	4 hrs.		
Hospital floor dust, R.T. dark	195 days	Garrod	1944
diffuse sunlight	Bactericidal		
Air in dormitory	0.22 inf. particles/cu. ft.	Green	1945
Air in movie	0.33 inf. particles/cu. ft.		
Air in school room	0.63 inf. particles/cu. ft.		
Air in recreation room	0.38 inf. particles/cu. ft.		
Infections appreciable only when premises occupied. Diminished to low level very quickly after vacation.		Green	1945

TABLE A12—(Continued)

The Survival of Streptococcus Species in the Air

Factor(s)	Survival	Reference	
INDOORS Continued			
Information relative to occurrence in air of hospital.		Hamburger	1944
Nasal carriers disperse 80–100 times as many strep. as do throat carriers alone.		Robertson	1947
Some influence of humidity on death rate as in pneumococci (10), but effect less pronounced.		Robertson	1948
S. pyogenes			
Talking		Robertson	1948
Relative dry air, saliva suspended, triethylene glycol vapor	Highly susceptible after 5 hrs. desiccation	Robertson	1951
Air, low RH, saliva, suspended in air, triethylene glycol vapor	Slower rate of kill after desiccating 20 h.		
Droplets from coughing	Practically none expelled	Robertson	1948
Blankets	>4 mos.	Robertson	1947
Floor dust	Several days	Lidwell	1950
Army barracks, air. Contamination high in spring, low in winter and summer. Bedding of persons with positive culture showed higher count. Highest counts obtained during maximum activity.		Loosli	1948
Air and dust in hospital wards	From war wound inf.	Miles	1940
Air in barracks during heavy activity	40/10 cu. ft. of air	Miller	1948
Air in barracks during moderate activity	2/10 cu. ft. of air		
On floor, petri dish, dark	20% alive, 14 days	Phelps	1939
diffuse light	<1% alive, <7 days		
Sprayed culture	Practically all settled in 48 hrs.		
Air, single noseblow by carriers	Millions recovered	Hamburger	1946
Air, coughing by carriers	Relatively few recov.		
Sneezing by carriers	Very few recovered		
Triethylene glycol, RH 40–50%, bed making	88.6–54% reduction	Hamburger	1945
Air, hospital wards, glycol vapor	Diminution of bacteria of 32–75% during periods of glycolization	Hamburger	1945
Air after shaking cloth	Persisted over 15 min.	Duguid	1948
S. pyogenes (Group C)			
Ultraviolet	In low conc. of air, UV markedly reduced bact.	Henle	1942
Freshly atomized	Not killed as fast as those in low humidity, floating for 5 hrs.	Robertson	1951
RH 15–20%, floating in air for 5 hrs.	Killed twice as fast as those exposed after atomization		
S. pyogenes (Group A)			
Bedding and floor dust	4 days	Lemon	1944
Air	378/cu. ft.		
Air, droplet nuclei expelled by sneezing	50%, 20 min.	Robertson	1948
S. salivarius			
Vapors of lactic acid, mandelic acid and triethanolamine, RH 70%, 15–21° C.	Gave good kills of org. in sprayed saliva	Lovelock	1944

TABLE A12—(Continued)

The Survival of Streptococcus Species in the Air

Factor(s)	Survival	Reference	
INDOORS Continued			
S. spp.			
Air, dust, R.T.	2 weeks	Phelps	1941
Dust	Long periods	Pressman	1937
Blankets and air	Many months	Robertson	1944
Glycol vapor	Caused 70% reduction in bacteria		
Room, floor and blankets treated with triethylene glycol	90% reduction	Robertson	1944
Survive better at low than at high RH.		Lidwell	1950
Dust, oiled linens and floors	Survive 19 wks. 92.3% reduction during bed making. 79.1% reduct. during sweeping	Bigg	1947
1st hour after cleaning	Found in air, not found after 2 hrs.	Furtherer	1946
Air drying	Many months	Ernst	1900
OUTDOORS			
S. pyogenes			
Air after shaking clothes	Persisted over 15 min.	Duguid	1948
S. spp.			
Air, drying	Many months	Ernst	1900

TABLE A13

The Survival of Viruses in Air

Factor(s)	Survival	Reference	
ALTITUDE			
Numerous viruses are carried along with dust into the atmosphere, many of which are not killed by light or heat.		Lange	1927
EXPERIMENTAL			
Influenza			
Vapors of lactic acid	Sterilized nebulized allantoic fluid infused with influenza	Catalano	1948
Moisture, stagnating moist air, ammonia, and other alkaline substances in the air increase incidence of grippe. Presence of acid substances in the air and dry clear weather counter-act grippe epidemics.		Cauer	1949
Dust, drying	Inoc. 1×10^4 after dry. Recov. 0, 3 wks.	Edwards	1941
Air of experimental tank	1 h.	Edwards	1943
Aerosol over water (PR8)	Reduction in infectivity 90%, 30 min.; 99%, 1 h.; 100%, 3 h.	Edwards	1943
Influenza virus dispersed in air is killed more quickly in humid than in dry air. Exposure to bright daylight increases the rate at which sprayed organisms die off in air.		Edwards	1943
56–57° C. for 30–45 min.	Necessary for inactivat.	Hirst	1943
Atomized susp., R.H. 50%	Death of 22.5% exp. mice	Lester	1948
Atomized susp., R.H. 30–80%	Death of 100% exp. mice	Lester	1948

TABLE A13—(Continued)

The Survival of Viruses in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL, Continued			
Atomized, dialysed susp. R.H. 50%	Death of all exp. mice	Lester	1948
27–29° C.,R.H. 80–90%	Infectivity time, 1 h.	Loosli	1943
27–29° C., R.H. 45–55%	Infectivity time, 6 hrs.	Loosli	1943
27–29° C., R.H. 17–24%	Infectivity time, 24 hrs.	Loosli	1943
Dust dried in floor sweepings (PR8)	22 hrs.	Loosli	1943
Vaccinia			
Chorio-allantoic memb. chick, sprayed in air	8 hrs. More susceptible to room envirn. than strep.	Buchbinder	1941
Virus retained virulence in all gases for 3 weeks when kept at 4° C. Became virulent at 37° C. Pure oxygen or carbon dioxide gas destroyed virus at 18° C.		Noguchi	1918
Susceptibility of virus to irradiation is of same order as bacteria.		Rivers	1928
INDOOR			
Influenza			
Sterile blanket	Survives drying	Krueger	1942
Influenza			
Dispersed into air (A)	Killed more swiftly in humid than in dry air	Loosli	1943
Dust	Long periods	Pulvertaft	1947
Mice exposed 20 min. (PR8)	Recov. in 0 mice, 20 min.	Robertson	1942
Air	Infected mice after 3 hrs.	Robertson	1943
Dust	Survived for days	Robertson	1943
Air, R.H. 80–90%, 1cc. triethylene glycol vapor	<1 h.	Robertson	1944
Air, R.H. 25–30%, 1cc. triethylene glycol vapor	36 hrs.	Robertson	1944
Air, dry, after shaking canvas floor covering	6 days	Robertson	1944
Air	Many days	Robertson	1944
Lab. conditions, 1cc. vaporized triethylene glycol	Highly lethal	Robertson	1949
R.T., R.H. 15–40%, vapor of triethylene glycol saturation 40–100%	Killed in 2–3 min.	Robertson	1949
R.T., R.H. 15–40%, vapor saturation of glycol 70–90%	Exposed mice completely protected against lethal concentration of virus.	Robertson	1949
Salt free virus atomized into an atmosphere of R.H. 50%. All mice died indicating that lethal effect of 50% humidity had been abolished by removal of salt.		Robertson	1948
Air, sprayed with glycol	40–60 min.	Robertson	1943
Dispersed into air, R.H. 20–30%, mice exposed	22% died at R.H. 45–60% death rate inc. to 80% R.H. when all mice died.	Robertson	1948
Type A. The per cent recovery of the virus aerosol was independent of initial concentration of atomized suspension. High aerosol recovery obtained in extreme ranges of 32–68% R.H.; minimum recovery 60% R.H. The mean diameter of influenza virus aerosol was <0.5 micra. Characterized by greater infectivity when introduced by air borne methods rather than intra-nasal routes.		Schechmeister	1950
R.H. 32–65% (A)	High aerosol recovery	Schechmeister	1950
R.H. 60%	Minimum aerosol recovery	Schechmeister	1950

TABLE A13—(Continued)

The Survival of Viruses in Air

Factor(s)	Survival	Reference	
OUTDOOR			
Foot and mouth			
Normal atmospheric cond.	1 week	Burbury	1928
Related epidemiologically to distribution in dust		Ostertag	1943
Infectious jaundice			
Dust borne dried excreta carries causative organisms. Less common in towns than rural districts.		Anderson	1947
Influenza			
Mucin in air (Type A)	Recov. 0, 15 hrs.	Parker	1944
Air current	Recov. 2, 72 hrs.	Parker	1944
Dried with talc in air	Recov. 0, 22 days	Parker	1944
R.H. 80–90%, 1cc. glycol vapor	<24 hrs.	Post	1945
Dry	36 hrs.	Post	1945
Smallpox			
Epidemic in India			
Humidity rise	Lower incidence of disease		
Low absolute humidity favors disease		Rogers	1928
R.H. low absolute	Seasonal rises incidence	Rogers	1948
GENERAL			
Air-borne viruses killed as easily as strep. salivarius by HCl0 mists.		Edwards Hollaender	1943 1943
Animal viruses have sensitivity similar to bacteria at wave length of 2537 Å.			
Means of obtaining infections in lab.: 1) by use of Waring blendor. 2) Opening sealed glass ampoule. 3) Inhalation of infectious material. 4) Inadequate disposal of contaminated material. 5) Inadequate handling of autopsy material.		Smadel	1951

TABLE A14

The Survival of Yeasts, Molds and Fungi in Air

Factor(s)	Survival	Reference	
ALTITUDE			
Alternaria			
Air	Found up to 16,000 ft.	Stakman	1923
Black stem rust spores			
Viable up to 10,000 ft. in air		Cotter	1931
Cronertium ribicula			
Air	Found 110 miles beyond limit of pines.	Pennington	1925
Aeciospores in air	Carried 300–400 miles in Pacific coast region.	Lachmund	1941
Fungi imperfecti	Found at 36,000 ft.	Rogers	1936
Air			
Gymnosporangium			
Air	Viable basidiospores found up to 2,000 ft. Good per cent viable several days.	MacLachlan	1935
Molds			
Found above 19,000 ft. in air			

TABLE A14—(Continued)

The Survival of Yeasts, Molds and Fungi in Air

Factor(s)	Survival	Reference	
ALTITUDE, Continued			
Pestalazzia			
Air	Viable spores at 18,000 ft. over Washington, D.C.	Proctor	1934
Phaeocrytopus gaumanni			
Spores		Meier	1933
	Blown across North Sea from England to Denmark.	Buchwald	1939
Puccinia graminis			
Spores	Blown from south into Dakota and Minnesota in 2 days	Lambert	1929
Spores collected at 10,000 and 16,500 ft. in Southern U.S. in spring.		Proctor	1942
Tritici carried 50–250 kilometers over sea without losing viability.		Roussakov	1926
Air, blown from South to Dakotas and Minnesota in 2 days.		Stakman	1934
Ragweed pollen			
Air during flights	Greatest conc. pollen at 3,500 ft.	Heise	1948
Rust			
Spores	From height of 5,000 ft. in 30 mile wind may travel up to 1,200 miles.	Proctor	1942
Spores	3 miles high	Stakman	1923
Sclerospora philippinensis			
Air	Viable spores travelled 8–80 ft.	Weston	1923
Uredeospores			
Found viable up to 5,000 ft. at Norway House, Manitoba, which was assumed that they had been carried horizontally for at least 200 miles.		Bailey	1928
Of leaf and stem rust	Found up to 10,000 ft.	Stakman	1923
General			
The influence of winds aloft on the concentration of solid particles is of minor importance compared to lapse rate.		Heise	1949
Four species isolated from Antartica where mean temp. is —20° C.		McLean	1918
EXPERIMENTAL			
Aspergillus			
Exposed to U.V.	10 min., innum. col., 20 min., 0 col.	Hart	1939
Geotrichum			
Exposed to U.V.	3 min., innum. col., 20 min., 0 col.	Hart	1939
Monilia			
Exposed to U.V.	1 min., 200 col., 5 min., 0 col.	Hart	1939
Mucor			
Exposed to U.V.	1 min., innum. col., 5 min. 25, 10 min., 0 col.	Hart	1939
Penicillium			
Exposed to U.V.	10 min.,innum. col., 20 min., 0 col.	Hart	1939

TABLE A14—(Continued)

The Survival of Yeasts, Molds and Fungi in Air

Factor(s)	Survival	Reference	
EXPERIMENTAL, Continued			
Pink yeast			
Water spray	Viable 10–14 days	Kirstein	1900
Saccharomyces albicans			
Ozone, 4 hrs.	60 seconds	Ransome	1901
INDOORS			
Fungi paralleled bacteria except more abundant in textile room with high R.H.		Matuso	1935
OUTDOORS			
Coccidioides			
Chlamydospores in dust. Easily transported in air.		Dickson	1938
Infection rates highest in dry seasons and dusty conditions. Rate of infection diminished by dust control. Southern climates have highest infection.		Smith	1946
Molds			
Spores in sea air	Long periods of time	Zobell	1942
Puccinia malvaccarum			
Moist atmosphere, 1° C.	50 days	Cohen	1946
Phytomonas malvacera			
Wind-blown rain	Important factor in dissemination	Faulwetter	1917
Sporotrichosis			
15–20° C., high R.H., abundant rainfall	These conditions must exist several days for contraction of disease.	Mackinnon	1949

SUMMARY OF ABBREVIATIONS USED IN TABLES

alk.	alkaline	org.	organism
avg.	average	path.	pathogenic
bact.	bacteria	physiol.	physiological
C.	Centigrade	ppm.	parts per million
col.	colonies	ppt.	precipitate
conc.	concentration	R.H.	relative humidity
cont'd., cont.	continued	R.T.	room temperature
ct.	count	recov.	recovered
cult.	culture	refrig.	refrigeration
d., ds.,	day or days	sec.	second
dessic.	dessicate	sensit.	sensitization
dil.	dilution	soln., sol'n.	solution
envirn.	environment	spp.	species
F.	Fahrenheit	str.	strain
fl.	fluid	susp., susp'n.	suspension
G.P.	Guinea Pig	T.B.	tuberculosis
gel.	gelatin	temp.	temperature
h., hrs.	hour or hours	U.V., UV	ultraviolet
inc.	increase	wks.	weeks
inoc., innoc.	inoculate	×	times
irrad.	irradiated	yr., yrs.	year or years
lg.	large	>	greater than
max.	maximum	<	less than
med.	medium	+	present; plus
met.	methyl	0	none
min.	minute or minutes	−	minus
mos.	months	±	plus or minus
mult.	multiplied		

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DESIGN OF MEDICAL EXPERIMENTS*

JOHN W. FERTIG

The following quotation is taken from Cornfield on "Statistical Relationships and Proof in Medicine" (1954): "The truth of the matter appears to be that medical knowledge (and, one suspects, many other kinds as well) has always advanced by a combination of many different kinds of observation, some controlled, and some uncontrolled, some directly and some only tangentially relevant to the problems at hand." The relevance of this quotation is immediately apparent when one thinks of the various types of evidence pointing to the relation between the use of tobacco and the occurrence of lung cancer, such as animal experiments, retrospective and prospective studies. We propose to discuss and illustrate some of the principles involved in the design of medical experiments. These experiments are generally of the prospective type, referring to the collection of data concerning events which have not yet occurred, in contrast to retrospective studies where data are collected about events that have already occurred. Although our comments are restricted to medical experiments, these do not differ fundamentally from laboratory experiments, except that in these latter it is usually easier to specify and control extraneous factors influencing the observations and to establish the validity and reliability of the observations and measurements.

Planning the Experiment

In general, the purpose of an experiment is to measure the effect of one or more procedures or treatments on experimental subjects by a comparison with some other treatment which may in fact be no treatment or an orthodox treatment. The general desiderata of a good experiment are the similar application of objective criteria to similar groups, which should be large enough so that effects having practical importance will be detected. Experiments will differ greatly as to how these broad requirements are satisfied.

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Before embarking on any experiment, a long planning phase is usually necessary. It is essential to have a clear specification of the experimental subjects to be used, of the objectives of the experiment, and of the treatments to be utilized. A statistician having some familiarity with medical data and not merely with statistical methods should be brought into the experiment at the very beginning. He should be a full-fledged member of the team with the opportunity of being throughly enmeshed with the details and not just with the arithmetic at the end. His contribution will come not merely in assisting with the analysis of the data at the end, but in helping with the detailed planning and with the routine conduct of the experiment. Often his queries and suggestions help the experimenter to sharpen the statements as to objectives, treatments to be used, measurements to be taken, and selection of groups to be studied.

The objectives of the experiment consist of a series of questions to be answered or hypotheses to be tested. Often a number of questions are put to a single experiment, but one should not be too ambitious and try to answer too many questions from the same experiment; otherwise, none may be answered satisfactorily. Variations in the form of extra questions can come in later experiments. A clear statement of the questions to be answered will also determine what measurements need to be taken and what precautions must be taken to avoid bias in the measurements. The measurements will be of various kinds, as blood pressure, gain in weight, living or dead, grade of impairment, and this will determine what records are to be kept.

The treatments must be completely defined, including such details as dosage and frequency of administration, and once agreed upon should be adhered to throughout the experiment. The purpose of the experiment will determine just what type of controls, if any, are necessary and what precautions must be taken to insure comparability of the various groups. Occasionally a control treatment may be eliminated, if its effect has been demonstrated in the past to be consistent with the same type of experimental subjects as used in the present experiment. This case does not occur very often, however. Obviously, if a control group is used, it must be an integral part of the experiment and subjected to the same general procedures of measurement and recording as the other groups.

The planning phase of the experiment should also give con-

sideration to the way that the results are going to be reported, what tables are to be compiled, how the data are to be analyzed, what comparisons and significance tests are going to be performed. Detailed consideration of these steps will also suggest what sub-groupings of the experimental subjects are appropriate and how large the groups may need to be so that any real effects of practical importance may be detected.

Increasing the Precision of the Experiment

Experimental subjects are variable, so that two subjects usually differ from each other even when given the same treatment. Because of this inherent variability, the effects of a given treatment will not be consistent and some uncertainty is thus introduced into the conclusions. It is this biological variability plus the lack of standardization of the various steps and measurements made in the experiment which produces the experimental error that may mask the effects of the treatments. A well-designed experiment attempts to minimize these experimental errors.

One way of reducing the experimental error is to use more homogeneous material, so that some of the more obvious variations are eliminated. Thus, in an investigation of the treatment of pulmonary tuberculosis with streptomycin, the type of case to be used was defined as "acute progressive bilateral pulmonary tuberculosis of presumably recent origin, bacteriologically proved, unsuitable for collapse therapy, age group 15 to 25." The answer is then limited to a precise and specific question. It must be remembered, however, that generalizations to other types of cases cannot safely be made.

The usual way of decreasing the experimental error is to increase the number of subjects utilized. It is essential, however, no matter how large the various groups being compared, that they be alike with respect to factors which are relevant for the measurements involved. This consideration usually casts doubt on experiments comparing a volunteer group with non-volunteers or a current series with that of an earlier time or of another place. Comparability is usually attained by some form of random allocation of the subjects to the various groups. While any specific random allocation may favor one or another of the treatments, it does so to an extent allowed for in the usual significance tests.

Another way of increasing the precision of the experiment is to group the experimental subjects so as to control extraneous

sources of variation, and so that the subjects to which the treatments are applied are more closely comparable. One obvious way, if it is possible, is to have each subject participate in all the treatments. Great reductions in the experimental error can often be accomplished in this way. The theory of design evolved to such a high degree in agricultural experiments offers us many guides in this direction (Cochran and Cox, 1950).

The precision of the experiment may also be increased by refining the measurements utilized. Quantitative measurements, such as urine sugar, duration of fever, are usually preferred to such evaluations as living or dead, cured or not cured, if they are equally relevant. In general, objective measurements or assessments, where the human observer is only incidental, are preferred to subjective measurements. Many medical experiments do, however, involve subjective measurements. In that case, certain precautions must be taken so that the observer does not change his scale of measurement or criteria from one treatment group to another. To insure this lack of bias on the part of the observer, various blindfold techniques are used. All of these techniques aim to have the observer make the measurements without knowing to which group the subjects belong, so that he cannot let his personal likes or dislikes enter into the observation. There are many examples of such techniques, such as reading the X-rays without knowing to which of the several groups they pertain, having the treatments given by one person and the measurements made by another, or by putting up the treatments in identical form and assigning them according to some code unknown to the person giving the treatments and making the assessments.

Lack of objectivity may result not only from a bias on the part of the observer, but also on the part of the observed. Consequently, the subjects may have to be blindfolded also, so that they do not know to which group they belong. This generally involves giving the control group a placebo. The general aim is to expose the control group to the same manipulations as the treated group except for the actual treatment involved. Obviously, there may be some cases where this is not feasible, for example, when treatment involves operation and removal of the thyroid. The control group cannot very well be given a blank operation. Many of the issues involved in avoiding bias on the part of the observer and subject are admirably discussed by Brad-

ford Hill in his papers on "The Clinical Trial" (1951, 1952).

Some of the issues involved and methods of resolving them may be illustrated by three small experiments in progress or recently completed at the College of Physicians and Surgeons of Columbia University.

An Experiment on Pulmonary Ventilation. Two treatments—100 per cent oxygen, and compressed air containing 21 per cent oxygen—are to be compared with respect to their effect on pulmonary ventilation in cases of emphysema of a certain type (Bell). Twenty-four patients are available for the study. One method suggested for making the comparison was to divide the group at random into two groups, assigning the compressed air to one group and the 100 per cent oxygen to the other. The division at random may be made by assigning numbers to each of the 24 patients and then drawing lots or by referring to a table of two-digit random numbers and selecting the first 12 numbers between 1 and 24 for the first group, the remainder constituting the second group. This random allocation does not insure that every patient in one group matches one in the other group with respect to weight, age, body-build, severity of disease, or other factors, known or unknown, determining ventilation, but it does make the two groups alike within chance limits allowed for in the significance test of the averages. In order to satisfy ourselves and to silence the critics, it is usually necessary to show that the method of allocation has in fact produced comparable groups. If the experiment is conducted in the form indicated, it may not be conclusive with such a small number of patients because of the large experimental errors due to the variation among individuals and the imprecision of the measurement.

The experimental error could be reduced by taking, for example, twice as many patients. But, if one were going to make twice as many measurements, it would usually be a much better plan not to seek more patients but to give both treatments to each of the original 24 patients. Both treatments can be given to the same patient after the lapse of a suitable time interval. In this way, each patient serves as his own control and the two groups would be identical with regard to a whole series of factors determining pulmonary ventilation. Because of the correlation between the pulmonary ventilation of the same individual at two different times, we may expect that this second form of the experiment will be more capable of detecting differences in aver-

ages than the first form using 48 different patients. Incidentally, it should be pointed out that the method of analyzing the data will change according to the design used.

In the second form of the experiment it is advisable to randomize the order in which the two treatments are given to the patients so as to balance out the effects of any possible residual carry-over from one treatment to the other, any possible learning effect with the techniques on the part of the patient or observer, or any steady progression of the disease. This may be done by a coin toss for each patient to see which treatment he is to be given first. This will insure within chance limits that each treatment is first half the time and thus that both treatments share equally in any change in extraneous conditions that might have taken place between the first and second reading. If it seems important, the randomization can be so arranged that each treatment appears first exactly half the time. This could be accomplished by drawing 12 numbers at random from 24 to decide which half of the 24 shall receive, say, compressed air first and 100 per cent oxygen second, and which half shall receive the treatments in reverse order.

In this experiment it is not necessary to perform the same operations on a group of normal individuals, if one is merely interested in contrasting these two treatments for patients with emphysema. If, however, one is trying to learn something about the mechanism of the disease, it may be necessary to perform a similar experiment on normal individuals, or on individuals with other diseases. There may be some difficulty in securing a group of individuals differing from the patients with emphysema only with respect to the disease in question. The disease may in itself involve certain physical concomitants, such as weight loss or general debilitation.

The form suggested for the experiment on pulmonary ventilation of letting the individual serve as his own control is a variation of the "before and after" type of experiment, for example, blood vitamin A for athletes before and after exercise. Here, however, we cannot randomize the order unless two exercise periods are involved. If the change may be due to factors other than exercise and we wish to examine the effect of the exercise only, it is evidently necessary to divide the group of athletes into two and subject one group to exercise, the other to all the maneuvers except the exercise.

An Experiment on an Anti-Vomiting Drug. A small scale experiment was performed on the effectiveness of a certain drug in the control of vomiting in cancer patients treated with a certain chemical compound (Gellhorn). A series of 40 patients was to be divided into two groups, one to be given the drug, the other a placebo similar in appearance. The investigator did not know which of the two treatments the patient had received until the end of the experiment, since the two types of ampules were supplied to him in a random order, the code sheet being kept secret. The supplying of the ampules in random order has the effect of randomly dividing the 40 patients into two groups. The percentages of patients vomiting in the two series were just barely significantly different.

It was decided to continue the experiment on a small scale to obtain more conclusive findings. Since the chemical compound was given more than once to many of the patients, it was decided that each of the subsequent patients receive both the placebo and the drug, one-half receiving placebo first, the other half the drug first, the halves being chosen at random. This is a before and after type of experiment where the end result is expressed as a percentage.

An Experiment on Radiation Sickness. A fairly large scale study was made to evaluate the preventive aspects of dramamine and pyridoxine against radiation sickness in cancer patients given X-ray therapy (Silverman, *et al.*). In addition to a control group which was given a placebo, the investigators decided to give a group a combination of dramamine and pyridoxine in the same quantities as administered separately. In this way two comparisons are available of the effect of dramamine, namely, dramamine against placebo and (dramamine plus pyridoxine) against pyridoxine. Similarly, two comparisons are available for pyridoxine. In addition, the difference in the two comparisons of the effect of dramamine (or pyridoxine) will yield information about the interaction of the two drugs, that is, whether the action of one differs according to whether the other is present or absent.

Considerable preliminary planning was necessary to standardize as much as possible the method of assessing the various symptoms and grades of radiation sickness among the participating clinicians, and to determine what records were to be kept. While the use of different clinicians in assessing the patients detracts somewhat from the precision of the experiment, in spite of the

attempt to standardize the criteria, no bias can develop thereby because of the random way in which the patients are assigned to the treatment groups. In view of the probable frequency of radiation sickness or of symptoms indicating radiation sickness and in view of the magnitude of the treatment differences which would be worth detecting, it was decided that some 200 cases be utilized—a two year program.

The patients to be utilized vary as to site of radiation and as to radiation dosage, particularly in relation to duration of radiation. While a division of the patients into the four treatment groups on the basis of a single list of random numbers should equalize the groups sufficiently well with respect to these factors, it was decided to force the equalization by making a separate allocation for each subclass according to treatment time (less than 8 days, 8–21 days, more than 21 days) and site (abdomen, thorax, head and extremities). There are thus 9 subgroups and consequently 9 small experiments. Although none of these subgroups will yield enough numbers to give adequate treatment comparisons, we do have the assurance that in the overall comparison the four treatment groups are alike as to these site and dosage factors. Within each of the 9 subgroups, the allocation was made independently by rotating at random the four treatments, using for this purpose the occurrence of the numbers 1 to 4 on the roll of a die (random numbers could also have been used). Thus, we might have a list such as 4, 1, 2, 3, 1, 3, 4, 2, 4, 3, 1, 2, etc. for one of the nine subgroups. Various comparisons among the groups made at the end of the experiment as to the initial status of the patient with respect to severity of disease, age, etc., showed that the various groups were comparable.

The list of numbers were prepared in advance and given to the secretary of the project who assigned the treatments accordingly, although the number itself was not recorded on the record sheet to avoid the possibility of any blind prejudice on the part of the clinician. The pharmacist made up four supplies of identically appearing capsules numbered 1, 2, 3, 4, the contents being unknown to the secretary or any of the investigators until the experiment was completed and much of the statistical analysis performed.

The fine scheme of equalization was slightly distorted by the fact that a few patients were withdrawn from the study because of discontinuation of the X-ray therapy during the first day or

two of the study. The number was small, however, and, because of the random allotment used, should have no prejudicial effect on the results.

The differences in the percentages of the various groups experiencing radiation sickness are relatively small and are not statistically significant. The results are given in the table below.

Drug	Number of Cases	Per Cent Sick
Pyridoxine	47	36
Dramamine	48	46
Placebo	46	48
Dramamine + Pyridoxine	44	54

It should be remarked that the data collected in the course of this study are hardly suitable for the comparison of sites as to frequency of radiation sickness, nor for a comparison of times, since the experiment was not designed for that purpose. The patients assigned to the longer times are necessarily different in severity from those assigned to the shorter, in addition to being composed differently with respect to the sites irradiated. This illustrates the fact that an experiment designed for one purpose cannot very well serve for some different purpose.

Summary

The main issues in the conduct of a medical experiment have been discussed briefly. These concern the detailed planning of the experiment in terms of objectives, treatments, measurements, and the construction of the groups. Great precautions must be taken to avoid bias in the composition of the groups and in the making of the measurements. These biases are usually avoided by some form of random assignment of the subjects to the groups and by the use of appropriate blindfold techniques for the observer and the subjects. The possibility of decreasing the experimental errors by an appropriate grouping of the experimental subjects is also briefly discussed.

Some of the principles are illustrated by three small experiments on pulmonary ventilation, anti-vomiting drugs, and preventives against radiation sickness.

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HYPERPLASIAS, METAPLASIAS AND ADENOMAS IN SENESCENT MALE RATS INTACT OR TREATED WITH HORMONES*

V. KORENCHEVSKY

As in the previous observations on development of adenomas in senescent rats (Korenchevsky, 1950, 1951; Korenchevsky and Paris, 1950, 1951), the actual aim of the present experiments was to study the effects of hormones on the processes of ageing. The gerontological part of the experiments will be published elsewhere.

In this paper are given only those results which were obtained without any intention to produce them, and which relate to the development of hyperplasias, metaplasias or adenomas in various organs of the intact senecent male rats and those treated with testosterone propionate or dipropionate, oestradiol benzoate-butyrate, cortisone acetate and desiccated thyroid (abbreviated in the text as testosterone, oestradiol and cortisone respectively).

At present, rats are often used in oncological investigations, while an accurate description and photomicrographic illustrations of hyperplasias and metaplasias, and their gradual transition into adenomas and other tumours are scarce or absent, especially in the case of some organs. Our present and previous papers contribute to the filling of this gap.

This appears to be important, since our results also indicate that the examination of the speed and extent of the development of minute nests of a few hyperplastic or metaplastic cells into benign or malignant tumors might be a valuable method in oncological research schemes related to old age. So far, however, comparatively little, if any attention has been paid to this method.

In our previous experiments (1950, 1951) it was observed that

1) with the onset of first senescence changes, *i.e.*, from the age of about 20 months, in the untreated rats of the breed used, the smallest nests of hyperplasias or metaplasias appeared in various

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organs; apparently these changes might be considered as one of the secondary features of ageing;

2) some of these nests, through the intermediate stages, developed into adenoma-like structures;

3) as compared with control animals, in the rats treated with sex and thyroid hormones and anterior hypophysis extract these nests and structures appeared to be more numerous, or some of them developed into larger structures or adenomas. Definite conclusions were handicapped by a comparatively small number of animals, not sufficient for oncological investigations.

The first two results obtained are in agreement with somewhat similar observations made in man. The literature on the subject was reviewed, *e.g.*, by Ewing (1940) and Willis (1948). They conclude that many simple hyperplasias in various organs, through the gradual intermediate stages, develop into benign adenomas, and can finally become malignant neoplasms. This was also observed in fishes (Lucke and Schlumberger, 1949).

The literature on spontaneous adenomas in ageing rats was reviewed in our previous papers, but the investigation by Yeakel (1947) was not referred to. She examined histologically the adrenals in 19 female and 18 male rats, 700 days old or older. Hyperplasias and adenoma-like structures in the adrenal medulla were observed in 16 per cent of the females and 72 per cent of the males, *i.e.*, there appeared to be a sex difference in the frequency of these formations.

Contradictory observations, however, were made on the role of endocrine factors in the incidence, development and treatment of tumours. A comprehensive review of the extensive literature on the subject was given in a monograph-symposium edited by Twombly and Pack (1947), and several relevant data were recorded by Burrows and Horning (1952), while a concise and clear summary of the discrepancies in the observations made was presented by Huggins (1949). Thus, while some investigations clearly showed the oncogenic action of certain hormones, in other experiments the same hormones produced various degrees of inhibiting or curative effects. Oestrogens produced mammary and uterine cancers in mice, but in women three altogether different procedures—gonadectomy, administration of androgens or treatment with oestrogens—were reported to ameliorate or regress the cancer of the female breast. In the cases of cancer of the breast in men, the androgens stimulated the growth of the

tumour, oestrogens produced it (simultaneously, however, more or less regressing the prostatic cancer), whilst castration apparently had a favourable effect. Castration had also a similar effect on the prostatic cancer, but increased in some strains of mice the incidence of "cancer produced by oestrogens" (Silberberg and Silberberg, 1952), spontaneous lymphomas (Murphy, 1944; Law, 1947), adenomas or carcinomas in the adrenals of mice (Woolley, 1949) and adenomas of these glands in guinea pigs (Spiegel, 1939).

Ingle (1950) reviews the literature on beneficial effects of cortisone on various tumours, chiefly of lymphoid structure. A temporary inhibition of the growth or a more or less pronounced regression of already developed tumours was observed in experimental animals and man. In contrast to these data, Cade (1955) records his own results and those of previous workers on the effects of bilateral adrenolectomy on the breast cancer in women. The life of operated patients was maintained by cortisone injections. For example, in the case of 56 women, adrenalectomised by Cade, a subjective and objective improvement was obtained in about 60 per cent, being "quite remarkable" in about 23 per cent.

Kirkman and Bacon (1952) showed that the oncogenic effect of oestrogens in producing renal tumours in male hamsters depends on the dosage of the hormone. Possibly the dosage factor might explain some contradictory results obtained by various authors.

Technique

Three experiments were performed on 176 male normal rats. The arrangement of the rats into groups, the number of rats in each group and the number of pooled hyperplasias, metaplasias, and adenoma-like structures or pure adenomas are given in tables 1–3. The age of the rats, the duration of the experiments, and the doses and combinations of the hormones used in the experiments were as follows.

Experiment I. 88 rats, aged 22–29 months, were used, being equally distributed according to their age in experimental groups. The duration of the experiment was 65 days. Hormones were given in the following doses:—testosterone propionate was injected as an emulsion of crystals in two doses, the larger dose 5 mg., the smaller dose 2 mg., both being injected during the first

TABLE 1

Experiment I. Number of meta-hyperplastic structures in organs of rats treated with sex and thyroid hormones

Groups of rats	No. of rats in groups	No. of rats with structures in						Average No. of liver structures in standard area
		Thyroid	Parathyroids	Adrenals	Pancreas	Thymus	Liver	
Controls	11	3	0	1	0	0	5	4
Des. thyroid—small dose	10	4	0	0	0	2	7	20
Des. thyroid—large dose	9	4(5)	1	2	1	2	5	14
Oestradiol B.B.	9	3	0	2	1	2	4	7
Testosterone prop.—small dose	10	3(4)	0	3	2	6	4	9
Testosterone prop. +des. thyroid—small doses	10	4(6)	0	2	0	.	5	5
Testosterone prop.—large dose	10	6(7)	0	6	0	.	5	4
Testosterone prop. +des. thyroid—large doses	10	7	0	3	1	.	5	13
All three hormones—large doses	9	0	0	0	0	.	3	4

In this and following tables the numbers in parenthesis in the "Thyroid" columns represent the total number of structures when counting every separate structure in each thyroid.

The thymuses were found in atrophic condition, with minute structures, only in a few rats of the last four ("testosterone") groups. In the remaining rats of these groups the thymuses and structures were completely atrophied.

TABLE 2

Experiment II. Number of meta-hyperplastic structures in organs of rats treated with cortisone acetate, testosterone propionate, oestradiol B.B. and thyroid hormone

	No. of rats in groups	No. of rats with structures in						No. of liver structures in standard area
		Thyroid	Parathyroids	Adrenals	Pancreas	Thymus	Liver	
Controls	8	3	0	1	2	2	4	5
Cortisone acetate	11	8(11)	0	4	3	4	7	5
Testosterone prop.	8	6(7)	0	4	3	2	4	10
Cortisone acetate +testosterone prop.	11	5(7)	1	5	2	2	9	7
Cortisone acetate +testosterone prop. +oestradiol B.B.+des. thyroid	8	5(6)	0	3	1	5	4	8
Cortisone acetate +testosterone prop. +oestradiol B.B.	3	2	0	2	0	1	2	5

TABLE 3

Experiment III. Number of meta-hyperplastic structures in organs of rats treated with cortisone acetate, testosterone dipropionate, oestradiol B.B. and thyroid hormone

	No. of rats in groups	No. of rats with structures in						No. of liver structures in standard area
		Thyroid	Parathyroids	Adrenals	Pancreas	Thymus	Liver	
Controls	11	2	1	0	1	2	2	3
Cortisone acetate.	6	3(4)	0	2	1	2	1	1
Testosterone dipropionate ...	7	3(4)	0	2	1	4	1	1
Cortisone acetate +testosterone dipropionate ..	7	3	0	2	1	0	0	0
Cortisone acetate +testosterone dipropionate +oestradiol B.B. +des. thyroid.	8	5(6)	0	1	0	3	0	0

5 weeks twice a week, and in the following weeks—once a week. Oestradiol B.B. was injected subcutaneously in oil, 0.046 mg. during the first week only. Desiccated thyroid was given as an emulsion in the 5 per cent gum acacia solution by pipette by mouth in a weekly dose of 85 mg. (smaller dose) or 160 mg. (larger dose) once a week during the first 6 weeks of the experiment; in the following weeks 85 mg. was given to both groups of rats once a week.

Experiment II. 49 intact rats, aged about 31.5 months, were used. The duration of the experiment was 67 days. Testosterone propionate was implanted as a pellet, consisting of 5 mg. of the hormone + 15 mg. of cholesterol. Average absorption from the pellet equalled 0.227 mg. per week. Cortisone acetate was injected as an emulsion of crystals, 0.3 mg. twcie a week; and 0.044 mg. of ostradiol B.B. in oil during the first week only. Desiccated thyroid was given by mouth, 25 mg. twice a week during the first seevn weeks of the experiment, and once a week during the last part of the experiment.

Experiment III. 39 rats, aged about 21 months, were used. The duration of the experiment was 45 days. Testosterone dipropionate was injected as an emulsion of crystals, 0.5 mg. every tenth day during the first half of the experiment, later—every fourteenth day. 0.06 mg. of oestradiol B.B. in oil was injected during the first week only. Desiccated thyroid was given

20 mg. once a week by mouth, but during the last four weeks 8 mg. once a week only. Cortisone acetate was injected as an emulsion of crystals, 1.5 mg. once a week during the first 30 days; during the last two weeks of the experiments—2 mg. once a week.

Thus, in contrast to Experiment I in which large doses of testosterone and des. thyroid were given, in Experiments II and III these hormones were administered in small doses. Cortisone was injected in Experiment III in a considerably larger dose than in Experiment II.

All organs were embedded in paraffin and stained with haematoxylin and eosin. The thyroids and parathyroids were cut serially, and therefore no structure, even the smallest one, could be missed. Only one adrenal in each rat was cut and stained with haematoxylin and eosin, and used for the count of the structures. The other adrenal was used for frozen sections and lipoid staining, and therefore was not suitable for examination of the structures. In each rat similar parts of all other organs were investigated.

Hormones were supplied by courtesy of Dr. K. Miescher of Ciba Ltd., and sterile pellets were prepared by Organon Ltd.

Experimental Findings

It is difficult to classify the various meta-hyperplastic structures into separate groups. There are several gradual intermediate changes from simple meta-hyperplasias (*i.e.* simultaneous metaplasia and hyperplasia of the epithelial cells) to small adenoma-like structures, and from the latter to the true adenoma. Moreover, even in some very small nests of meta-hyperplasias, consisting, for example, of three thyroid follicles, some malignant-like features of growth can already be observed, namely an invasion of the metaplastic cells, by breaking follicular walls, into the neighbouring follicles (Fig. 1). Therefore, as it was previously (1950–1951) emphasized, even the smallest meta-hyperplastic nests of cells apparently might be considered as the latent quiescent centres of neoplasms. Accordingly, as in previous papers, in the following tables 1–3, all the gradual transitions from simple meta-hyperplasias to adenomas are pooled together and termed as meta-hyperplastic structures, but they are separately discussed in the text.

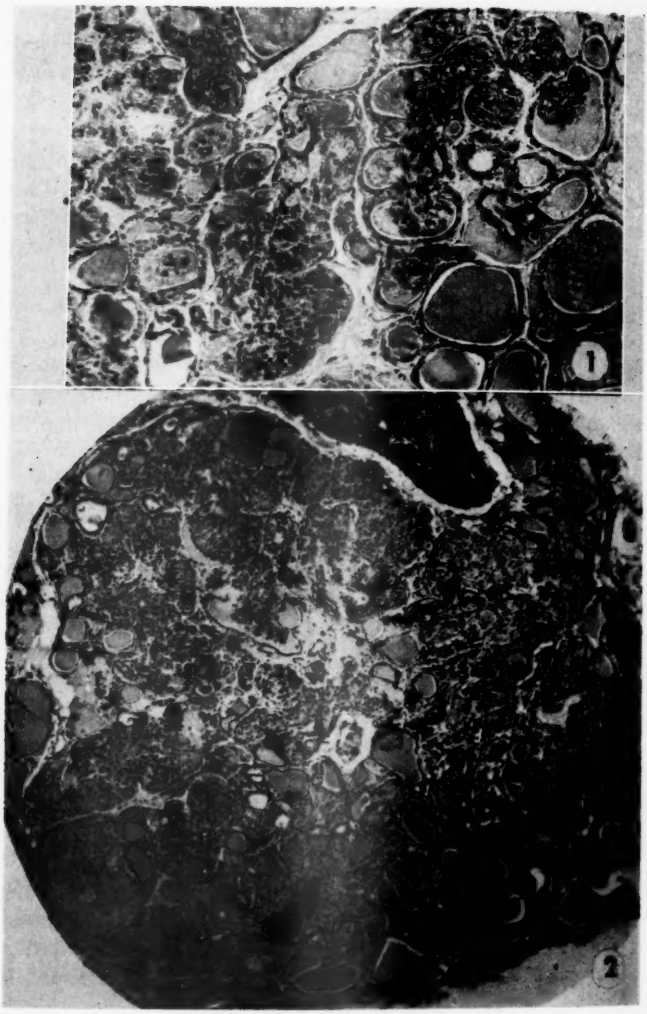

PLATE I. Thyroid

FIG. 1. Simultaneous beginning of adenomatous metaplasia in several follicles of the thyroid. In the right half of the figure—metaplastic cells break through the walls of four follicles. Rat from Exp. I treated with a smaller dose of testosterone. X 140.

FIG. 2. The only large size adenoma so far found in the thyroid of the control rats; dark body on the top—a part of the parathyroid. X 63.

Although in these tables the incidence of the structures in all organs investigated was given, their total accurate count was possible only in the case of the thyroid and parathyroids, since only they were cut and examined serially. Accordingly, in the following, these structures will be discussed in detail. In the unexamined parts of other organs some structures might be present and therefore missed in counts. Since in these organs, however, the same parts were always examined, the respective average figures have certain comparative significance, and their photomicrographs appear to be of definite value.

Only those varieties of structures were illustrated, the photomicrographs of which were not given in previous papers.

Incidence of Meta-Hyperplastic Structures in Untreated Control Rats

Although percentage figures for small numbers of animals in the groups are of doubtful significance, they might suggest probable relationships, especially if the range of variations is small, and similar data are repeatedly obtained.

Thus, the average incidence of the thyroid structures in 30 control male rats of the present experiments and 13 control male rats of the previous experiments (1950) was 26.7 and 23.1 per cent respectively. The respective figures in the case of the adrenals were 6.7 and 7.7 per cent; in the case of the liver—36.7 and 53.8 per cent; for the parathyroids—3.3 per cent and none; for the thymus—13.3 and 30.8 per cent. Therefore, these figures were close in the case of the thyroid, adrenal and possibly parathyroid structures.

A comparison between the above given incidence of the structures in control males of the present and previous experiments, and this incidence in older control females in the previous experiments (1950, Table 2) shows that the structures appeared to be more frequent in males than in females: *e.g.*, in the adrenals of the latter no structures were found, while in the thyroid they were present only in 5.9 per cent. This appears to confirm the above mentioned observation by Yeakel on the greater frequency of the adrenal structures in males.

Meta-Hyperplastic Structures in Treated Rats

1. *Thyroid structures*

They were subdivided into eight groups according to their size. For the latter purpose they were measured with an eyepiece

micrometer with the gauge dividing the whole field of the microscope approximately into 82 squares. The number of the squares, which were covered by the largest cross-sections of the thyroid structures, defined the following eight sizes of these metaplastic structures: I—from 1 to 2 squares; II—from 3 to 7 squares; III—from 8 to 15 squares; IV—from 16 to 25 squares; V—from 26 to 50; VI—from 51 to 100; VII—from 101 to 230; VIII—from 231 to 700, or larger. In some rats the thyroid contained two or exceptionally three structures, usually one in each lobe of the thyroid. This total number of structures was also counted, and in the "thyroid columns" of the table it is represented by the figures in brackets.

The percentage of rats with structures in the three control groups of the present experiments was 18.2, 27.3 and 37.5 respectively. As compared with this, in the treated groups the percentage of rats with structures was 40, 42.9, 60 and 75 in the "testosterone" groups; 40 and 70 in the "testosterone + des. thyroid" groups; 50 and 72.7 in the "cortisone" groups; and 42.9, 45.5, 62.5, 62.5 and 66.7 in the "cortisone + other hormones" groups.

These figures appear to suggest some stimulating effect of the above mentioned hormones or their combinations on the development of structures. This, however, was not corroborated by the measurement of the size of the structures. To begin with in the present control groups, in contrast to the previous experiments one comparatively large adenoma (size VI) was recorded. Although the largest sizes VII and VIII were found in the treated groups, they occurred in five rats only (in three of "testosterone" groups and two of "cortisone + testosterone" groups). The number of the structures of other sizes was approximately about the same as in the control groups. In a group treated simultaneously with testosterone + des. thyroid + oestradiol B.B. (Table 1, last line) no structures were observed at all.

There were three new forms of adenoma-like structures or adenomas, which did not occur in our previous experiments.

Thus, papillomatous cysto-adenomas were found in the thyroid of 12 rats. These also develop gradually from small structures (Fig. 5) through intermediate stages into cysto-adenomas occupying the whole thyroid and considerably enlarging it (Figs. 3 and 4).

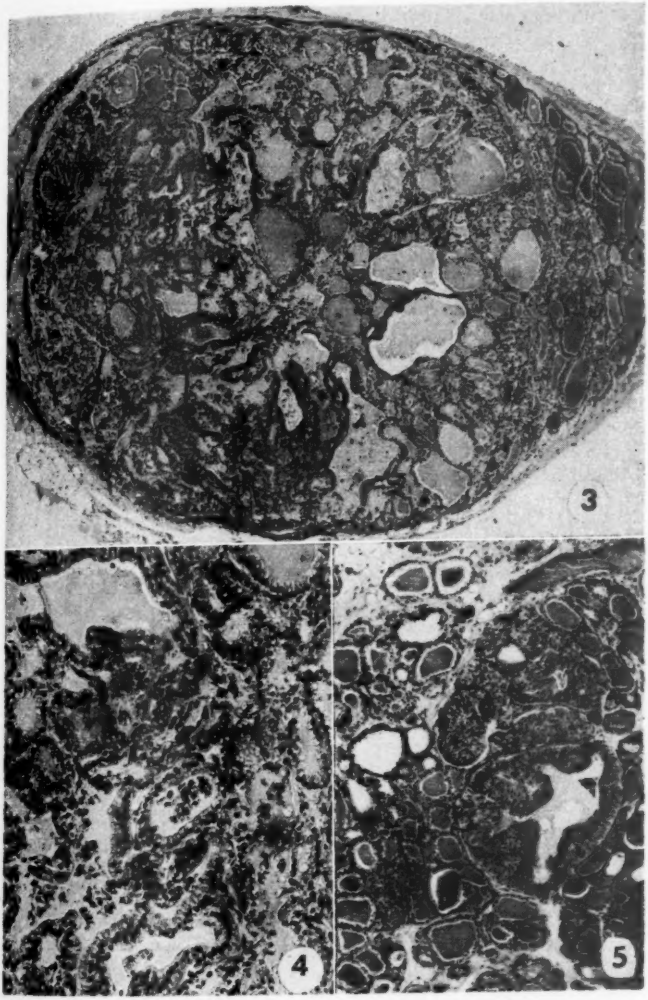

PLATE II. Thyroid

FIG. 3. Papillomatous cysto-adenoma in the thyroid of a rat from Exp. I, treated with large dose of testosterone propionate. X 43.

FIG. 4. The same as in figure 3, but at X 140.

FIG. 5. The beginning of the development of a cysto-adenoma in a control rat. X 63.

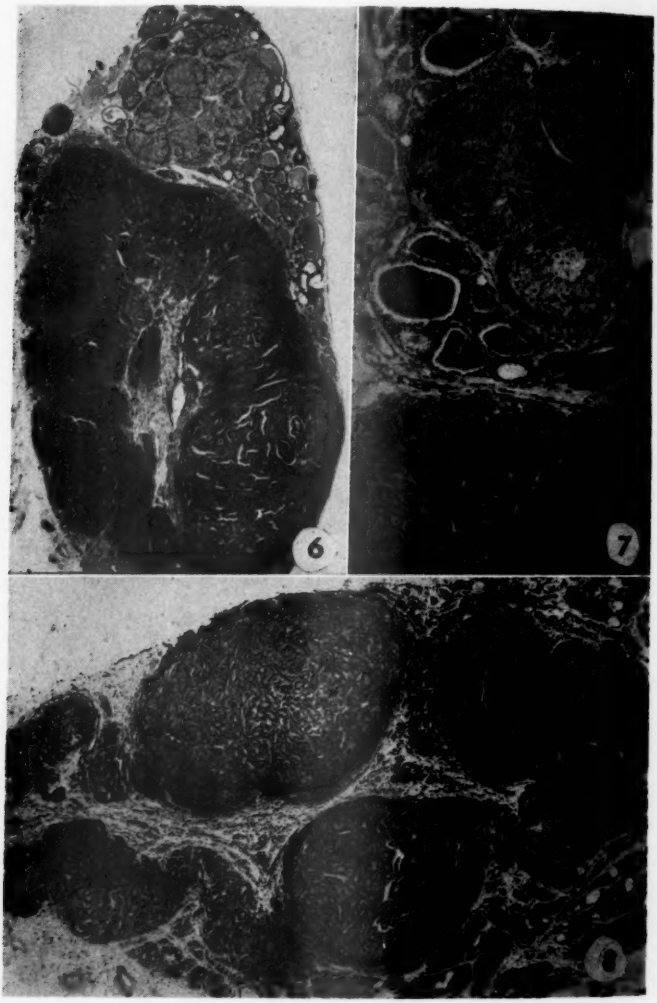

PLATE III. Thyroid

FIG. 6. The thyroid of the rat treated with very small dose of testosterone propionate, from Exp. II. It contains two adenomas of different structure: the top one with light large cells and vesicular nuclei; the bottom one with small cells and nuclei, and a more darkly stained cytoplasm. Whilst the origin of the first is clearly from thyroid cells, the origin of the second tumour is uncertain, whether from the thyroid or parathyroid cells. X 21.

FIG. 7. Border line of the same adenomas as in figure 6, but at X 90.

FIG. 8. Adenoma in a rat treated with cortisone acetate and testosterone propionate, from Exp. II. It invades the fibrous tissue surrounding the thyroid. X 28.

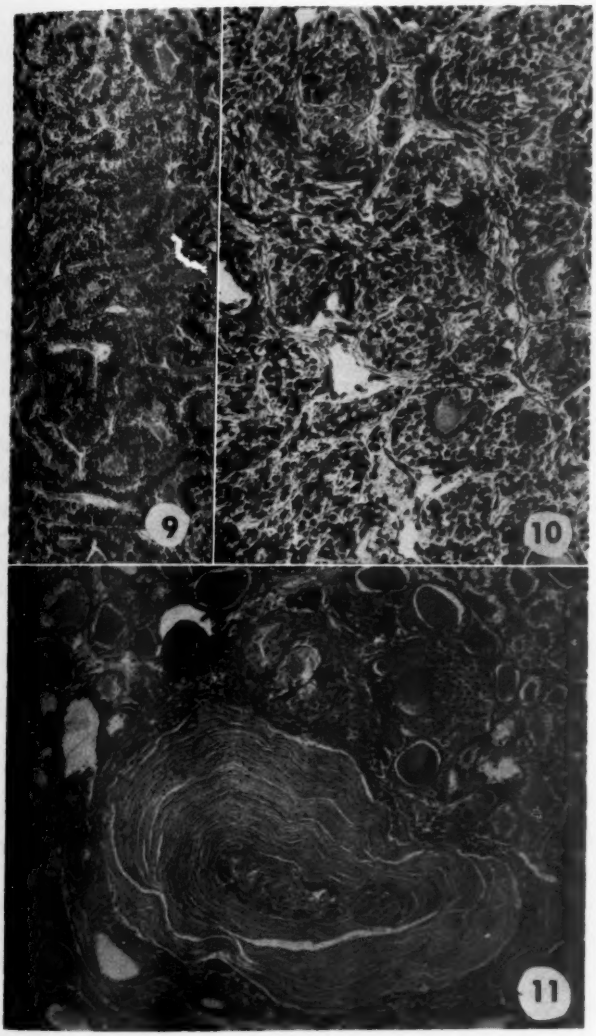

PLATE IV. Thyroid

FIG. 9. The same adenoma as in figure 8, but at X 140.

FIG. 10. Thyroid adenoma from a rat in Exp. II treated with a small dose of testosterone. Disorganised growth and form of cells, in places resembling sarcoma cells. X 140.

FIG. 11. Rat treated with cortisone acetate from Exp. III. Starting small adenoma with squamous metaplasia in the centre. X 90.

The second form consisted of tubular adenomas and is represented by figures 6–9. They were included in the tables as the probable thyroid structures. It was difficult, however, to establish with certainty whether these tumours developed from the thyroid or parathyroid cells. They occurred in three rats. The tubules had no lumen in one tumour; and in two others the lumen contained a transparent colloid-like secretion (Figs. 6, 7, 9). A striking difference between these and the usual thyroid adenomas is clearly demonstrated in the case of one thyroid, in which both types were simultaneously present (Figs. 6 and 7). In two cases, containing tubular adenomas, no parathyroids were found in serial sections. In one case, however, a typical parathyroid was present. In rats, in some cases, each lobe of the thyroid contains, besides the main parathyroid, also one or rarely more small accessory parathyroids. Therefore, the presence of the parathyroid, in one of the three above-mentioned cases, does not exclude the possibility of the tumour developing from the accessory gland.

In one case (Fig. 8) a tubular adenoma apparently penetrated into neighbouring connective tissue. By mistake, however, when dissecting, the tumour was cleaned from the neighbouring tissues; and therefore it is impossible to say how far the ingrowths of the tumour occurred into these tissues.

The third form of adenoma-like structures was observed in three rats. It contains squamous metaplastic centre or centres in the otherwise usual type of structures (Fig. 11). This form did not reach a large size in our experiments.

2. *Parathyroid structures*

In the preceding section on the thyroid structures, in the treated rats the special tubular adenomas were described and their possible origin from parathyroid cells was discussed.

Besides these adenomas, a nodular meta-hyperplasia of the parathyroid, considerably enlarging this gland, was observed in one rat treated with testosterone and cortisone. It was divided into smaller nodules by fibrous trabeculae (Fig. 12.)

There was also a very small nodular hyperplasia occupying about one-twelfth of the parathyroid in one control rat, and a small meta-hyperplasia, occupying about one eighth of the gland, in another rat treated with thyroid hormone. In the previous experiments (1950–1951) only one typical parathyroid adenoma was observed with two types of cells in a rat simultaneously

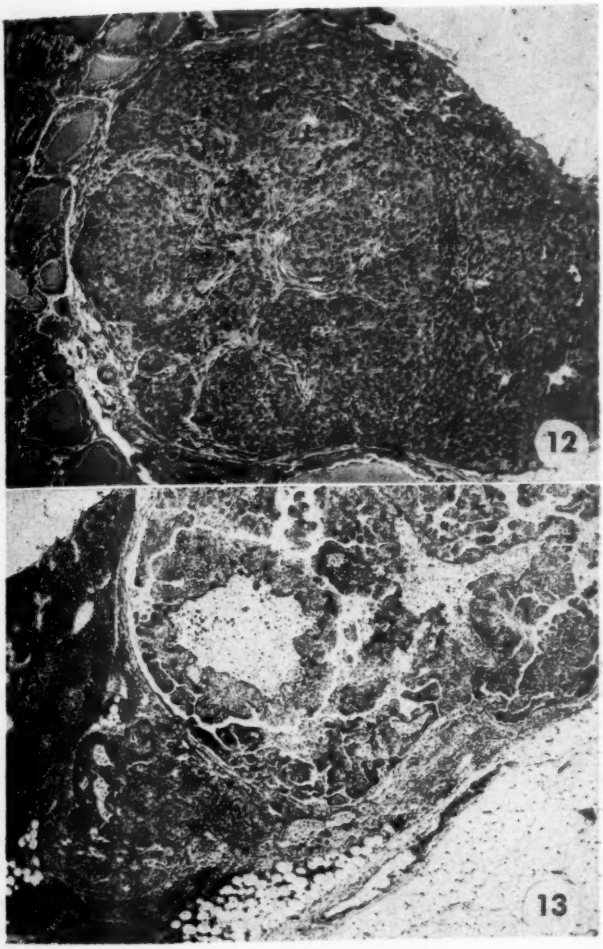

PLATE V. Parathyroid and Thymus

FIG. 12. Nodular meta-hyperplasia in the parathyroid of a rat from Exp. II treated with cortisone acetate and testosterone propionate. X 95.

FIG. 13. Cysto-adenoma of the thymus (thymoma) in a rat from Exp. II, treated with cortisone. Narrow stripe of the normal thymic tissue in the left hand edge of the photograph (dark, with closely packed dark nuclei). It is invaded by thymoma (light, with wider situated lighted nuclei). X 35.

treated with des. thyroid, progesterone and oestradiol B.B. These cells and their arrangement were altogether different both from tubular adenomas and also nodular hyperplasias found in the present experiments.

3. *Adrenal structures*

In the previous investigation an increased incidence of the structures, and in most cases an increase in their size, were observed both in normal and castrated rats treated with testosterone propionate, oestradiol B.B., des. thyroid and desoxycorticosterone acetate administered singly, or when des. thyroid and oestradiol were given simultaneously. When, however, all four hormones were administered simultaneously, not a single structure was found in this group of rats (1950, Table 3).

In most groups of the treated rats (Tables 1–3), the incidence of cortical structures was again increased in various degrees, and in some rats they were of a larger size, except the group in which three hormones were given simultaneously (Table 1). The same negative result was obtained in this experiment in the case of the thyroid structures (Table 1, last line). Not a single structure occurred in these groups.

If these negative results are confirmed on a large number of senescent rats, the effect of the simultaneous administration of certain doses of certain hormones on the development of the adenomatous structures might be different from, or even opposite to, that produced by a single hormone.

The gradual development of small nests of the cortical meta-hyperplasias into adenomas and their breaking through the adrenal capsule was followed in the present experiments as easily as in our previous investigation. In some cases the middle parts of the adenomas consisted of the foamy cells with the vacuoles of various sizes. Various structures of other cortical adrenal adenomas was similar to those which were previously observed and illustrated. For example, in one rat a large extra-capsular adenoma was spreading over the adrenal, as in the case which was illustrated in figures 21 of the previous paper (Korenchevsky and Paris, 1950).

Castillo and Sammartino (1938), in the experiments of long duration on 13 rats injected with large doses of oestradiol benzoate, found three adenoma-like hyperplasias and one adenoma in the adrenal cortex, *i.e.*, in about 30.8 per cent. This figure is

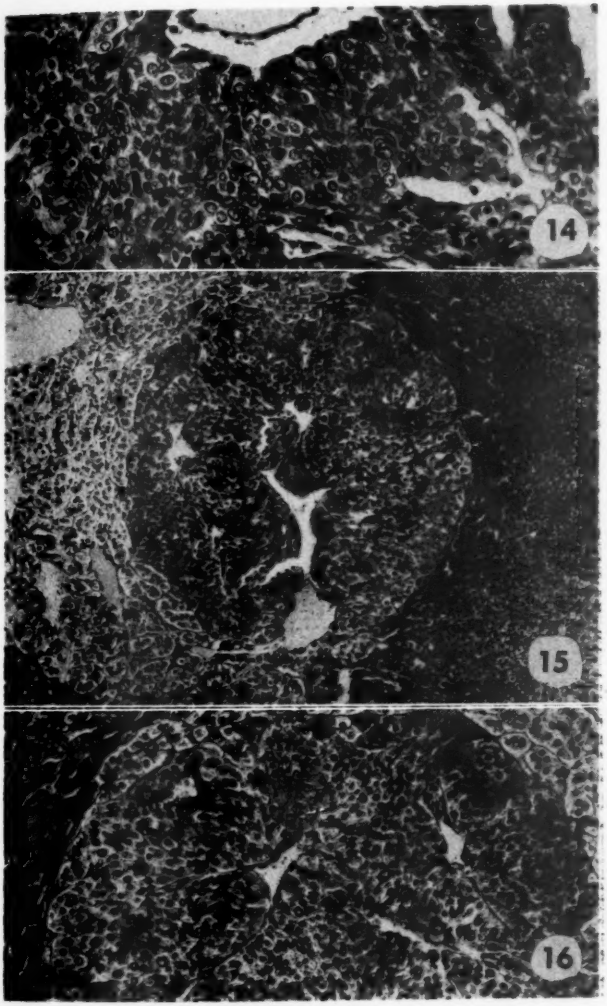

PLATE VI. Thymus and Adrenal

FIG. 14. The same thymoma as in figure 13, but at X 280. Great variability of the cellular and nuclear form and size.

FIG. 15. Nodular meta-hyperplasia of the adrenal medulla, surrounded by normal medullalar and z. reticularis tissues. Rat from Exp. III, treated with cortisone. X 90.

FIG. 16. The same as in figure 15, but at X 145. Various sizes and forms of cells and their nuclei.

within the range of the corresponding figures in our present (22.2 per cent) and previous (33.3 per cent) experiments on male rats.

In our previous and present experiments the extra-capsular cortical adenomas started from the cells of z. glomerulosa, the intracortical adenoma—usually from the fasciculata cells; but in their growth the gradual adenomatous transformation of all cortical zones was involved.

Small adenoma-like structures in the medulla of the adrenals were observed in only two cases, one in a rat treated with cortisone, the other in a rat treated with testosterone. The larger structure is illustrated in figure 15. The cells and nuclei were of various sizes and forms (Fig. 16).

4. *Thymic structures*

The meta-hyperplastic structures of the thymus were described, and the literature on the subject was given, in the previous papers (1950–1951).

In the present experiments, in control rats, the structures were absent in Experiment I, but they were present in about 18–25 per cent in the II and III experiments (Tables 2–3). They were very small, occupying only a small part of the thymic lobule. In the first of these rats the structure was solid, in the second rat—cystic, and in the third rat it consisted of a few small nests or ribbons of epithelial cells.

In the treated rats of Experiment I the incidence of the structures appeared to be increased, especially in the "small dose of testosterone" group. The "large" dose of this hormone, however, was so great that in the treated rats the thymuses were either completely atrophied, or in the greatly atrophied glands the structures, although present, were very small. They were surrounded by fibrous capsules and remnants of the atrophied thymic tissue. In the previous paper (Korenchevsky, 1950) photograph 7 illustrates well these testosterone structures. Larger structures were observed in Experiments II and III, especially in the former. They were of the spreading type as illustrated previously (Korenchevsky, 1950, Fig. 6), or in the form of compact thymomas. A part of such a large thymoma, occupying most lobules of the thymus in a rat injected with cortisone acetate, is illustrated in the present paper by figure 13. This thymoma appears to invade into the normal thymic tissue, and was of papil-

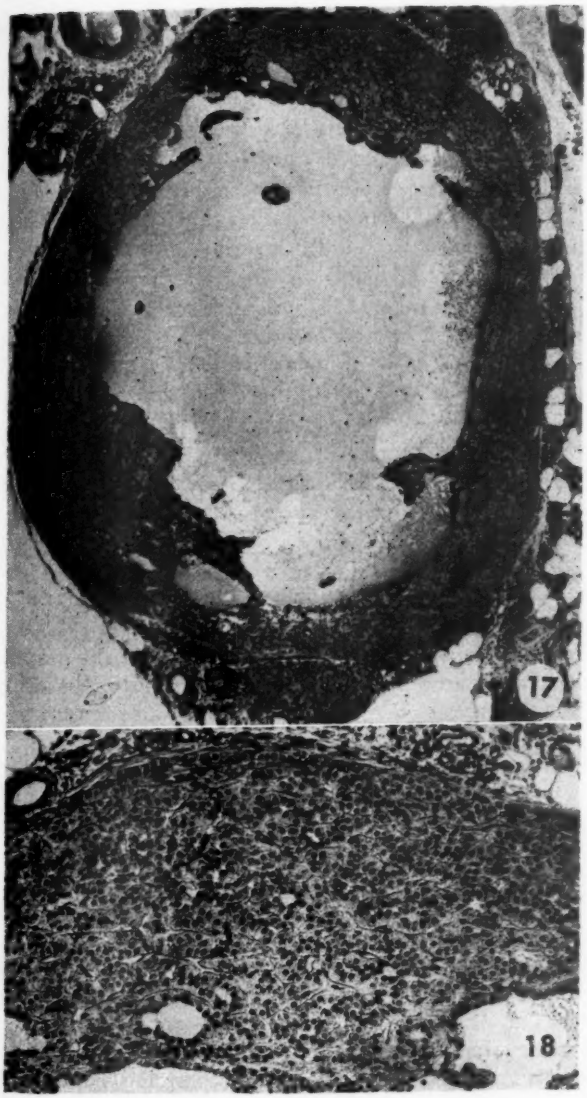

PLATE VII. Pancreas

FIG. 17. Meta-hyperplasia of an Island of Langerhans, with formation of a cyst in the centre. Control rat. X 45.

FIG. 18. The same as in figure 17, but X 140.

lary cystic type. Its cells and nuclei were of various sizes and shapes, in places spreading in irregular arrangements (Fig. 14).

5. *Pancreatic structures*

In the present experiments the incidence of the pancreatic structures in most groups was too small for comparisons.

Besides developing from the exocrine acinar cells, the structures originated also from the smallest pancreatic ducts. This was observed previously and their histology was described in the previous papers (1950, 1951).

Two cases of adenoma-like hyperplasias, developing from the Islands of Langerhans, were observed in the present experiments. One was in the control group with the formation of an inside cyst (Figs. 17 and 18), another of about the same size but of solid structure—in the testosterone group. These structures were larger than those previously observed.

As in the previous experiments, no additive cumulative effect of hormones on incidence or size of the structures was recorded.

6. *Hepatic structures*

Bile ducts hyperplasias in senescent female and male rats were previously described and illustrated (1950, 1951).

As in previous investigation on male rats, in the present experiments the same indefinite effect of hormones on the incidence and development of bile duct hyperplasias was recorded.

7. *Testicular structures*

In the previous experiments the histological examination of the testes was completed after the publication of the paper. Only in one intact male rat, 20 months old, which received 19.3 mg. of des. thyroid three times a week for 68 days, a nodular hyperplasia of the interstitial Leydig cells was found (Figs. 19 and 20). In the present Experiment II, in the testis of a rat simultaneously treated with cortisone, testosterone, des. thyroid and oestradiol one large (the size of a small pea) and one small tumour were found (Fig. 21). Both were of the same structure, with the cells and nuclei similar to, but smaller than those in the case of the nodular hyperplasia. The cytoplasm was similarly vacuolated in both cases, which suggests that the origin of these tumours was from the Leydig's interstitial cells. At the perhiphery of the large nodules, of which the tumour consisted, the cells were smaller

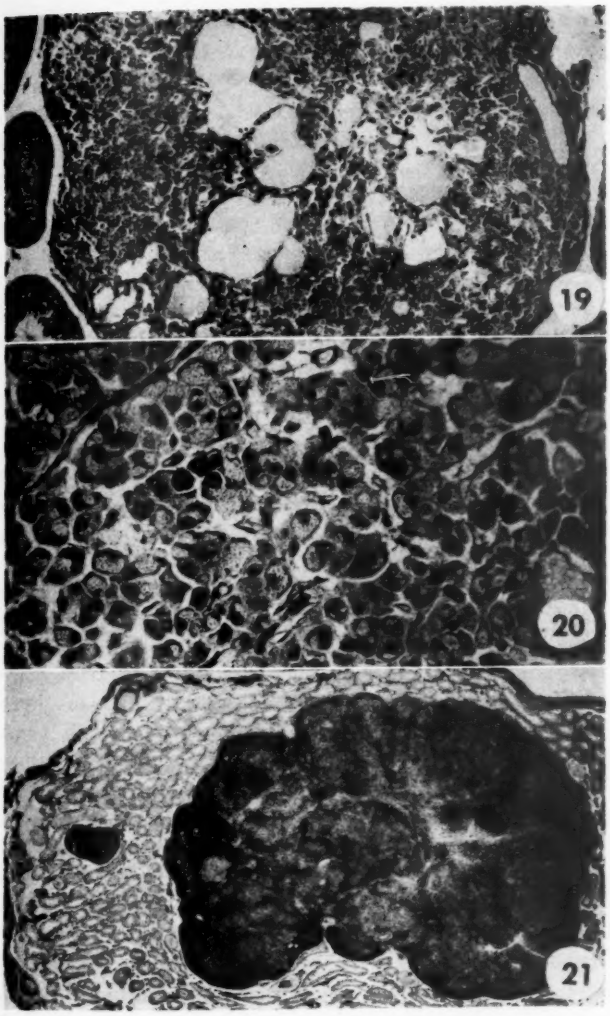

PLATE VIII. Testes

FIG. 19. Nodular hyperplasia of Leydig cells in the testis of a rat treated with des. thyroid. X 63.

FIG. 20. The same as in figure 19, but X 280.

FIG. 21. One large and one small adenoma of the testis, both of the same structure and with cells similar to those in figure 20. X 13.

and more closely packed, and a small amount of lymphocytes appeared to be present in these areas.

No hyperplasias or tumours were found in the control rats.

Saxton *et al.* (1948), found the interstitial cell tumour in only one rat out of the 198 examined. However, the hyperplasias and tumour originating from these cells were often observed in dogs (*e.g.*, Huggins and Pazos, 1945), but seldom in other animals and man. Willis (1948, p. 580) emphasizes that "no sharp distinction can be made between nodular hyperplasia and early neoplasia of the Leydig cells." Bonser and Robson (1940), Hooker *et al.* (1940), and Shimkin *et al.* (1941–42) produced them in certain strains of mice by the administration of oestrogens. The literature on the subject was well reviewed by the above-mentioned authors, and Gardner (1947).

Discussion

It has been already emphasized that the results obtained by previous investigators were often contradictory. The main deficiency of our previous and present experiments is that they were designed for study of certain processes of ageing, and not for investigation of the oncologic problems. From this point of view, the number of animals in our previous and present experiments (total 365 male rats) was small, while the doses varied, and the duration of the experiments was too short, the longest one being 91 days. Also serial cuttings of all the organs investigated ought to be made, while we were able to do it in the case of the thyroid and parathyroids only.

It is also necessary to remember that the tumours are produced in various percentages in various strains of animals even by such definite oncogenic hormones as oestrogens. Within the strains there might also be some variations. Several instances of all these variations were quoted, *e.g.*, in the book of Twombly and Pack. It was also clearly shown by Bonser and Robson (1940) in the case of three strains of mice treated with oestrogens.

All these variations require long duration experiments on large numbers of animals for definite oncological conclusions. Therefore such conclusions on the effect of hormones used could not be drawn from our observations until similar experiments are performed on a large number of senescent rats.

In general, the results obtained in present experiments were less definite than those in our previous observations. For example,

while in our previous experiments no large adenomas were found in control rats, in the control animals of the present experiments one comparatively large adenoma in a thyroid, and a considerable adenoma-like hyperplasia of an Island of Langerhans were found. Then, although in some of the treated groups the total number of all sizes of metahyperplastic structures, *e.g.*, in the thyroids, was larger, the number of medium or small sized structures was about the same as in the controls; and only a few structures in the treated groups exceeded the largest size in the controls. If the hormones and doses used would produce a clear stimulating effect on the development of the structures, apparently it would be expected that not only their incidence, but their size as well should be increased.

In some cases of the previous and present experiments, when three or four hormones were given, there were no additive effects on the incidence and size of the structures. Sometimes, on the contrary, this effect was considerably decreased; or, as in some cases of the thyroid and adrenal structures, no structures were found. If confirmed on a large number of animals, this apparently paradoxical effect might be explained by the inhibiting effect of certain doses, or by the neutralising action of one hormone upon some other. The age factor in the onset and development of certain experimental and human tumours, in particular carcinomas, is well known. At the same time the above mentioned investigations of previous authors established the relation between endocrine factors and the development or regression of tumours both in animals and human beings. Our results suggest also relationships between age, endocrine factors and tumours. The apparent discrepancies, however, in several results obtained indicate that these relationships are of a complicated nature, because hormones appear to possess simultaneously both oncogenic and anti-oncogenic properties. Their selective action is probably determined by various conditions of experiments (*e.g.*, dosage, deficiencies in the endocrine system, age, sex and species of animals, etc.)

A striking difference in the action, even opposite effects, produced on organs and their function by small and large doses are already well known in the case of several substances (*e.g.*, thyroid hormone, some amino acids and purines, cytocoxic sera, arsenic). Therefore the effects of various doses and combinations of hormones on the structures might be different, and this might

explain some of the contradictory results, obtained in various, in particular our own investigations. It appears also probable that a greater incidence and development of tumours, perhaps their malignant transformation, would be obtained if experiments were of longer duration.

Summary

1. In three experiments on 176 senescent normal male rats, intact or treated with sex, thyroid or corticoadrenal hormones, various types of adenoma-like structures or true adenomas, developing in different organs from the smallest nests of hyperplastic or metaplastic cells, were described and illustrated.

2. In control intact rats of the present experiments the incidence of these structures in the thyroid, parathyroids and adrenals was similar to that observed in the same groups of male rats of our previous experiments.

3. The assay of the incidence and of the various developments of meta-hyperplastic formations in senescent rats appears to be a very suitable method for the investigation of relationships between endocrine factors, tumours and old age; but long duration experiments on large numbers of animals are necessary for definite conclusions.

4. The number of rats used in our previous and present experiments was not sufficient for such conclusions.

5. However, the results obtained by previous workers and those recorded in our previous and present experiments suggest that there are certain relationships between endocrine factors, tumours and ageing processes, but they are of a complicated nature so far not investigated properly.

6. These results also suggest that hormones might possess simultaneously both onco- and antioncogenic properties, the selective action of which probably depends on doses of hormones used and on some other conditions as discussed above. A complete separation of these two opposite hormonal properties and the possibility of using them selectively appear to be urgent aims of endocrino-oncological research.

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DRUGS AFFECTING MOOD AND BEHAVIOR*

CHAUNCEY D. LEAKE

About a third of a century ago the great German toxicologist Louis Lewin (1850–1929) published a remarkable book which was later translated into English under the title "*Phantastica: Narcotic and Stimulating Drugs; Their Use and Abuse.*" This volume reviewed the long and interesting social history of indigenous drugs used by different peoples throughout the world for exultation, or for psychological effects in religious rites. It also gave as much chemical, pharmacological, and clinical detail as was available regarding the active principles involved. Included were considerations of fermented beverages, such as beer and wine, used from antiquity, distilled liquors, as introduced in the Middle Ages, cannabis, opium, coca and coffee derivatives, tobacco, and hypnotic or anesthetic agents. In addition the action of peyote and of various ordeal poisons was discussed.

This book created a sensation among pharmacologists and sociologists, and it has become a rarity. Valuable was its influence in calling attention to the important considerations revealed by anthropologists in their study of the social uses of exhilarating or stupefying drugs. Clearly emphasized were the social regulations regarding the use of such types of drugs, as imposed by tabu or religious ritual. Similar to this volume by Lewin, but restricted geographically, is the recent *Las Plantos Fantasticas de Mexico* by Professor Francisco Guerra and Doctor H. Olivera.

Meanwhile exhaustive and detailed scientific studies were being made, following the introduction of modern chemical and pharmacological methods, of the characteristic actions and metabolism of alcohol, anesthetic agents, caffeine, cannabis, cocaine, hypnotic agents, and the opium derivatives, in particular morphine. The social problem of drug addiction, especially with morphine, cocaine, and alcohol, resulted in many comprehensive reports.

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* From the Pharmacology Laboratory of the Ohio State University Health Center, Columbus, Ohio. Presented before the Palo Alto Medical Research Foundation and the Institute for Behavior Studies, Stanford, California, July 26, 1955, and at the Peralta Hospital, Oakland, July 28, 1955.

With the extensive use of amphetamine, the possible behavioral effects of a synthetic central nervous system stimulant were partially explored. The attempt to develop synthetic nonaddictive morphine derivatives for pain relief has not been particularly successful. However, N-allylnormorphine was found to be a significant morphine antagonist.

Within the past few years the dramatic introduction of Rauwolfia compounds from ancient Hindu sources, for lowering blood pressure, and for tranquilizing disturbed patients, as well as the development of the quieting effects of the synthetic phenothiazine antihistaminic, chlorpromazine, focused attention again upon the importance of chemical agents in regulating behavior. The startling discovery that lysergic acid diethylamide could artificially induce characteristic psychotic states, was immediately recognized as offering an important tool for the study of the etiology of psychoses.

As a consequence of recent rapid developments in regard to drugs affecting behavior, there have appeared stimulating hypotheses about the significance of certain aspects of brain metabolism in psychotic states. In a practical way there have come valuable drugs and procedures for use in the management of mental disorder. It may be pertinent to attempt to analyze some aspects of this situation on which there may be reasonable evidence. A frankly speculative and over-simplified discussion of some of the chemical factors involved may aid in orientation for a consideration of possible practical clinical applications.

It is interesting to note the fluctuation of opinion regarding the etiology of mental disorder. Even the ancient Greeks in the Hippocratic School, had the courage to express the belief that "the sacred disease" (epilepsy) is no more "sacred" than any other disease, but that it has a physical origin, and is capable of treatment by physical means. We are still a long way from fully justifying this faith. As Price emphasizes, however, scientific flirting with the supernatural remains tempting, as in concern for "extraordinary perception."

For centuries insanity was associated with witch-craft as a supernatural phenomenon, capable of being handled only by superstitious and magical means or by restraint and "punishment." Zilboorg has given the long and dismal history of the gradual appreciation of controllable human factors in relation to mental disease. An optimistically inclined person might feel

that the slow and gradual improvement in the management of insanity is a tribute to the power of increasing human understanding and affection.

The philosophical duality of "mind and matter" still plays a large role in psychiatry. Even though Freud considered the possibility of chemical factors in the neuroses, his followers in general have not been sympathetic to such an idea. Now, with remarkable suddenness, a number of different lines of investigation seem to be converging toward realization of the probability that complex physico-chemical enzymatic metabolic processes involving ordinary bodily constituents may, if blocks occur, result in symptoms of mental disorder ranging from neuro-muscular withdrawal, as in schizophrenia, to neuro-muscular convulsive discharge, as in epilepsy. Practically, drugs are now available which seem to be able to alter such metabolic disturbance so that more normal behavior occurs. Indeed the situation is so encouraging as to inspire lay editorializing urging large-scale support for coordinated research between chemists, pharmacologists and clinicians in the hope of a break-through in the wall of ignorance about mental disease. This could result in effective rational management of what is our most important health problem.

Metabolic Hypotheses in Psychoses

From the facts accumulating by direct experiment with hallucinogenic drugs, and by analogy with symptoms in clinical psychoses, metabolic hypotheses on the etiology of psychoses are inevitably developing. The various steps in the formulation of one, involving faulty metabolism of adrenaline and noradrenaline, have been detailed in pleasing honesty by Hoffer, Osmond, and Smythies. This assumes the accumulation, under some faulty enzymatic metabolic process concerning adrenalin, of a hypothetical toxic intermediate product which causes schizophrenic symptoms.

Considering the well known matter of the gradual deterioration of adrenaline solutions, with the development of pink color on exposure to air, as a result of the formation of quinones, it was postulated that one of these decomposition products might be worth testing. There is clinical evidence that deteriorating solutions of adrenaline may cause psychic disturbances when injected intravenously in patients.

One of these quinones, adrenochrome, was synthesized and studied. It is a very unstable compound and on solution in animals will raise blood pressure. As the semi-carbazide it has even been used in surgery. No psychic disturbances result from this combination.

Hoffer, Osmond, and Smythies, however, administered adrenochrome itself in solution to human subjects with startling effects. In distilled water it causes great pain on injection, but when administered with the subject's blood the pain is reduced. In relatively small doses the psychic effects are similar to those produced by mescaline, which is 3, 4, 5-trimethoxy-phenyl-ethylamine, chemically related to adrenaline. It is the active principle of peyote *(Anahalonium Lewinii)*, which has a long history of use among the Amerinds of the southwest of the United States as an exultation agent in religious ceremonies. It was studied by Havelock Ellis and Weir Mitchell. Peyote addiction among the Navajos is now a serious problem. Experimental mescaline psychosis was well analyzed by Guttman.

On the basis of the direct finding that adrenochrome produces significant psychic changes in human subjects, and on the assumption that it is a metabolic product of adrenaline, Hoffer, Osmond, and Smythies postulate that schizophrenic conditions are caused by faulty metabolism of adrenaline, resulting from some disturbance in its enzymatic decomposition, so that significant quantities of adrenochrome may accumulate to produce the symptoms observed.

If this hypothesis is correct, Hoffer, Osmond, and Smythies point out that adrenochrome would be the first naturally occurring agent to be found in the human body capable of producing psychiatric changes. It has been reported that adrenochrome is present in small quantities in the brain, and even in muscle, under ordinary conditions.

Adrenochrome is not a phenalkylamine, but a quinone and an indole derivative. In the metabolism of adrenaline by amine-oxidase, or phenalase, it is assumed that the quinone structure is established, and that the pyrol ring is closed, to make the indole nucleus.

It is rather remarkable that many compounds having an indole or similar nucleus show marked central nervous system effects. These include such alkaloids as those of harmala, yohimbine, ibigaine, voacangine, lysergic acid diethylamide, 5-hy-

droxytryptamine, and even, by slight extension in similarity, such agents as cocaine, atropine, morphine, and related compounds, strychnine, scopolamine, physostigmine, and the xanthines, such as caffeine.

The significance of the indole group as a possible factor in the etiology of psychoses has been further emphasized by Wooolley and Shaw. They call attention to certain neurophysiological aspects of 5-hydroxytryptamine.

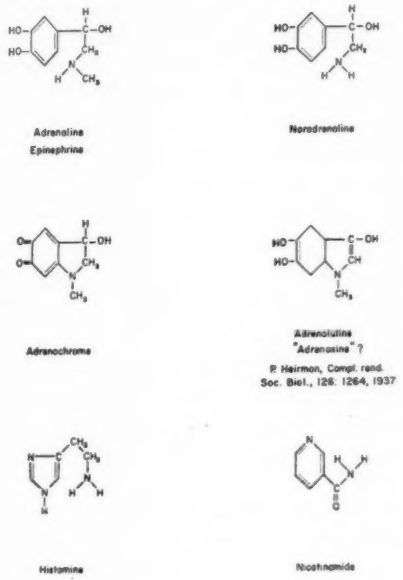


Fig. 1

The exciting story of 5-hydroxytryptamine has been reviewed thoroughly by Page and also by Erspamer. The latter details the long process by which the pressor agent in intestinal mucosa ("enteramine"), and the pressor agent in serum ("serotonin") as studied so well by Page and his associates, have both been identified chemically as 5-hydroxytryptamine. The direct administration in large doses of this compound experimentally may produce drowsiness, and marked psychic changes, in addi-

tion to various autonomic responses. Gaddum suggests that the preservation of the normal pattern of metabolism of 5-hydroxythryptamine is essential for sanity.

Woolley and Shaw consider the possibility that faulty metabolism of 5-hydroxytryptamine, which is a normal constituent of healthy body tissues and fluids, may occur with consequent changes in autonomic activity and in function. Wisely they postulate the possibility of faulty metabolism of 5-hydroxytryptamine resulting either in a deficiency of a necessary metabolite, or on the other hand in the accumulation of an excess of 5-hydroxytryptamine with consequent symptomatology. As they point out, the evidence favors an interference in metabolism of 5-hydroxytryptamine in such a way that an excess of the compound accumulating in the body may cause symptoms similar to those found in the major psychoses.

These are provocative hypotheses. They suggest many types of experimental procedures which may be used to obtain data which may be interpretable in respect to these ideas. Marrazzi and Hart have indicated some of the direct experiments that may be performed to investigate the validity of these hypotheses. Feldberg and Sherwood have reported pertinent methods with startling findings in experimental animals.

Woolley and Shaw suggest that many naturally occurring indole or related compounds, which do produce central nervous system effects, may act as anti-metabolites with respect to 5-hydroxytryptamine. They may compete with the enzyme systems responsible for the metabolism of 5-hydroxytryptamine in such a way as to allow the accumulation of 5-hydroxytryptamine with resulting symptoms. This competition may occur in various parts of the body, with effects of particular interest on muscle and nerve.

Here it is important to keep in mind always the vast complex of chemical reactions which occur in all parts of the body, but with particular rapidity and with remarkable energy transformation in nerve and muscle. The many complicated enzyme systems which are involved may be presumed to be highly sensitive to pH, and to redox potentials existing in the particular tissue at different times. These factors themselves result from the vast complex of vitamine, enzyme, coenzyme, hormone and electrolyte reactions, which tend to preserve the homeostatic condition

of the body fluids and tissues, and thus to maintain normal function.

Direct experimental evidence regarding the action of some of these naturally occurring body hormones, when given so as to come directly in contact with the brain stem, has been furnished by Feldberg and Sherwood. They extended preliminary exploratory experiments of this sort made by Cushing. Cushing found marked autonomic response as a result of the direct application of physostigmine and similar drugs to the lateral ventricle. Feldberg and Sherwood systematically applied drugs into the lateral ventricle of unanesthetized cats, and carefully observed the resulting response by moving picture.

With twenty microgram doses in Tyrode's solution, acetylcholine causes initial retching and vomiting, with subsequent quietness, withdrawal. and failure to respond to ordinary stimuli. Yet, on intravenous injection of larger doses in humans, convulsions may occur. Adrenaline in the same amount causes retching and vomiting with resulting effects similar to a light anesthesia resembling that induced by pentabarbital. 5-hydroxytryptamine in 100 microgram dose applied in the same way causes drowsiness, loss of muscle tone, salivation, and rapid respiration.

Adenosine triphosphate in a dosage of 200 micrograms given in the same manner gives effects similar to those induced by adrenaline or by 5-hydroxytryptamine. Atropine in the same dosage produces retching and vomiting. followed by increased liveliness. and increased responsiveness. Interestingly D-tubocurarine in the same dose causes increased muscular tone with subsequent epileptic convulsions. These can be partially prevented or counteracted by adrenaline. Di-isopropyl-fluorophosphate, like bulbocapine, is followed by a face-washing routine, and a catatonic stupor. In large doses acetylcholine will also cause a catatonic stupor.

These findings suggest many problems involving the passage of chemicals from blood to brain tissue, and bring up a plethora of questions about possible enzyme and energy transformations which may occur in the underlying chemical *milieu*. They do indicate however the clear possibility of significant findings by direct experimental investigation.

Electroencephalographic studies in connection with the symptoms resulting from these various agents indicate changes in the

electrical activity of the cortex which can be correlated roughly with the character of the general symptoms produced.

Of the utmost importance in studies of this sort, particularly if they are conducted in humans or extrapolated to humans, is the matter of cross control. Lasagna, Felsinger, and Beecher have indicated the role of underlying psychological moods existing in human subjects in connection with responses to drugs inducing changes of mood. Even placebos may be followed by marked changes of mood in susceptible subjects. On the other hand, Fischer in his study of model psychoses, has indicated the remarkable correlation between the extent of fixation by sulphur containing proteins of hallucinating drugs, and their relative effectiveness in producing psychic changes in humans.

The extraordinary importance of relatively simple factors in producing central nervous system effects may be illustrated by carbon dioxide. The inhalation of carbon dioxide up to 30 per cent, with oxygen, so that there is no question of oxygen want, produces extraordinary changes in catatonic schizophrenic patients. While these are of a very transitory nature, they indicate the extreme sensitivity of the chemical equilibrium existing in the human central nervous system and involved in behavioral effects.

On the other hand, R. J. Williams calls attention to the wide range of individual variation in metabolic pattern in different persons as reflected in vitamin "requirement" or "balance." He suggests that these variations may be involved in the marked behavioral differences observed even under similar training or conditioning circumstances, as among different siblings in the same family and environment.

Taking into account the huge number of complicated factors which seem to regulate healthy nervous activity, there still are the stubborn facts about the chemical make-up of drugs that alter mood and behavior. All of them seem to contain basic groups, either a free or substituted hydroxyl or amino group separated from each other by three, four, or five carbon atoms. This is a characteristic feature of the indole derivatives. It is the case with such tranquilizing agents as reserpine, chlorpromazine, and "frenquel." It occurs in hallucinogenic compounds such as lysergic acid diethylamide, 5-hydroxytryptamine, mescaline, harmine, and yohimbine. The occurrence of this sort of nucleus in histamine may have more than coincidental significance, in view

of the extraordinary effects produced by histamine on all types of cells. Hoch reports indications of helpful effect from histamine in some schizophrenics.

Similarities to this structure occur in many other types of naturally occurring alkaloids and synthetic derivatives which produce behavioral effects. This chemical arrangement is found in d-tubocurarine, as well as in strychnine, and even in morphine. On the other hand. it is not to be found in cannabinols, methenesin, or 2-methyl-2-n-propyl-1, 3-propanediol dicarbamate ("Miltown"), and it is not characteristic of the usual run of anesthetics, or hypnotics. Perhaps different mechanisms of action may produce similar over-all behavior or mood effects.

Some aspects of the mechanisms of action of neurotropic drugs have been discussed by Toman. Unfortunately this discussion was written before the exciting recent studies on hallucinogenic and tranquilizing drugs. Fischer emphasizes the importance of what he calls "LSD-tails," found in lysergic acid diethylamide. These constitute the chemical nucleus to which reference has been made, of -N-C-C-C-C-N-. The number of carbon atoms may be three or four, both conditions occurring in lysergic acid diethylamide. In methylene blue, which is a well known dye for nerve tissue, the "LSD-tails" both include four carbon atoms. These "LSD-tails" are found in most of the hallucinogenic drugs.

In this intricate complex of related chemical compounds with strong but varying effects on the activity of the central nervous system, surprising modification of action may occur from relatively slight chemical change. For example, it was long ago noted that uniform curariform action results in alkaloids affecting the autonomic nervous system if the tertiary nitrogen compound is changed to the corresponding quaternary ammonium form (Crum-Brown and Fraser, 1868).

Every opportunity exists for extensive experimental investigation in this fascinating field of biochemorphology, relating chemical structure to biological activity, with a slow accumulation of verifiable data, from which general principles of great importance may be derived. Emerging from the information already available it is clear that the response of the autonomic receptor organs to these various drugs may be quite similar, with marked differences, however, in effect on mood and behavior.

On this point, one must keep in mind the influence of feedback from peripheral organs and muscles to the central nervous

system, with resulting effect. It is well known that muscle relaxation helps to produce sedation, while increased muscle tension promotes alertness and wakefulness. Many of the effects of these various drugs on mood and behavior may be separately related to the character of feed-back from the peripheral response.

In considering the consequences of peripheral feed-back on mood and behavior, one may recall the significance of the "blood-brain barrier." Nobelatent W. B. Cannon emphasized the role of adrenaline in preparing the mammalian organism for flight or fight. This was in regard to the peripheral action of the hormone. Here a consequence of increased muscle tension is feed-back to the brain, increasing alertness and rapidity of adaptive response. Adrenaline, however, when applied directly to the brain causes lassitude, relaxation, and diminished responsiveness. Whatever the blood brain barrier may be, it effectively protects mammals from this sort of reaction when flight or fight are in order (some animals, like the oppossum and armadillo, do "withdraw"). Maybe the blood brain barrier is a chemical mechanism for such rapid oxidation of compounds like adrenaline, that effective concentrations cannot reach the neurons or synapses in the brain. If there is disturbance in the metabolic processes at this point, in certain individuals, adrenaline or its decomposition products may reach the brain, with resulting inappropriate or withdrawal behavior in the face of danger or stress. This is frank speculation, but it may afford a basis for obtaining facts that may aid in understanding.

Fischer indicates that certain phases of Selye's adaptation syndrome in stress are involved in schizophrenia. This suggests a way by which hypophyseal, adrenal cortex, and electrolyte factors may be involved in mental disorder.

In a series of recent brilliant reports, Brodie and his capable associates summarize results of studies on 5-hydroxytryptamine. Using fluorescent spectroscopy and bioassay methods, they find that the brain content of 5-hydroxytryptamine in cats and dogs varies from 1.7 gamma per gram of fresh tissues in the hypothalamus to 0.07 in the white matter of the cortex. This distribution corresponds to that of monoamine oxidase, the enzyme which metabolizes 5-hydroxytryptamine. Reserpine acts by releasing 5-hydroxytryptamine, which appears as indole acetic acid in the urine. After reserpine, the 5-hydroxytryptamine content of brain tissue falls to about 10 per cent of normal and slowly returns to

normal, the half-life of formation being about 10 hours. No other psychomimetic drug has this effect of reserpine, but lysergic acid diethylamide reduces the potentiation of alcohol or barbital by reserpine. Further, the antibacterial durg, "marsilid" (1-isonicotinlyl-2-isopropylhydrazine), which blocks the enzyme, monoamine oxidase, also prevents the release of 5-hydroxytryptamine.

Brodie postulates 5-hydroxytryptamine as a neuro-humoral agent, which acts on nerves by depolarization, and which has to disappear from the environment of the nerve cell and axone, in order to allow repolarization to occur, and thus for normal function to resume. Marrazi and Hart find that 5-hydroxytryptamine strongly inhibits synaptic transmission. One might postulate that central nervous system stability and equilibrium depends on the equilibrium between intracellular and extracellular 5-hydroxytryptamine in the brain and brain stem. A relative intracellular excess would seem to tend toward alertness, tenseness, and convulsive release, while a relative extracellular excess would apparently induce depression and withdrawal.

From the chemical standpoint the possibility of interference with the metabolism of naturally occurring hormones, such as noradrenaline, or 5-hydroxytryptamine, are many and varied. It would be a great contribution for some physical chemist to calculate the various competitive affinities of reactive groups in these compounds, such as the hydroxyl, the amino, and the methyl groups. Under the complex circumstances existing it is possible for many reactions to occur chemically, with the formation of agents capable of producing intense effects on mood and behavior. That such possibilities do exist is apparent from the fact that there is such a marked difference between individuals with respect to variations in mood and behavior. Evolutionary, inherited, and environmental factors play a great role in the shaping of the various interactions in this vast complex. In order to promote the worthy purpose of trying to get optimum mental health for as many people as possible, wise physicians will try to understand something of the general principles of the chemistry, physiology and pharmacology of the nervous system.

Practical Aspects of Mood-Altering Drugs

The standard texts in pharmacology and on therapeutics give the pertinent practical clinical information on the appropriate use in sick patients of such mood-altering drugs as alcoholic

beverages, hypnotics of many differing types, analgesics, both of the morphine type which relieve traumatic pain and of the salicylate and the aminopyrine type which relieve congestive pain, amphetamine, caffeine, and tobacco. They also give important clinical information on addictive drugs such as opium derivatives, cocaine, and marihuana.

As current reports appear on new hallucinogenic or tranquilizing drugs, the clinical data will be evaluated by the Council on Chemistry and Pharmacy of the American Medical Association, with resulting monographs which may appear in *New and Non-Official Remedies*. Wise physicians will wait for these reports before using these new drugs, unless the risks of frank clinical experimentation are assumed.

As is well known among physicians alert to psychomatic relationships, mood and behavior are profoundly altered by the physical condition of the patient. Insight and self-understanding are more readily obtained in patients with problems of an interpersonal or environmental nature, if nutrition and physical condition generally are good. Especially as patients get older is it wise to provide better nutritional status through relative increase in proteins (in which tryptophane and methianine may be critical factors) and in vitamines.

For optimum nervous stability, thiamine, nicotinic acidamide, pantothenic acid, and ascorbic acid seem to be critical vitamine factors. Nicotinic acid and ascorbic acid are very important in maintaining optimum cellular oxidative processes. Large doses of nicotinic acid or its amide are found to be clinically helpful in psychoses. Here it is interesting to note again the occurrence of the -N-C-C-C-C-N- nucleus. Panthothenic acid and thiamine seem to be directly concerned in preventing degenerative changes in the nervous system.

Whatever may be the relative importance of physical and psychological factors in mental disturbance, the wise physician will in his counselling, or by his example, follow the Hippocratic injunction to try to get the "externals" to cooperate, whether these "externals" be nutrition, physical surroundings, family, friends or associates. Fortunately now several new tranquilizing drugs may aid practicing physicians in more satisfactorily obtaining favorable response from mentally disturbed patients. These new drugs are briefly and incompletely reviewed herewith.

Rauwolfia

The oldest used of the newly appreciated mood-altering drugs is Rauwolfia. The roots, leaves, and juice were used from primitive times by the Hindus, for seizures, insanity, insomnia, dysentery, and fevers. This apocynaceous plant was named *Rauwolfia serpentina* by Plumier in the 18th Century, in honor of Leonhard Rauwolff of Augsburg, a pioneering 16th Century physician and botanist. In 1931 Sen and Bose found that clinically the powdered root reduces high blood pressure and controls insane manias.

In 1931 Siddiquis had isolated five different alkaloids from Rauwolfia root, but these have little pharmacological action. In 1952 Schlittler and Muller isolated the alkaloid, reserpine, and Bein observed its striking hypotensive and sedative effects. In a skillful and artistic manner, the details of *The Rauwolfia Story* have been published in brochure form by Ciba Pharmaceutical Products of Summit, New Jersey. This contains the pertinent references.

Wilkins made the first clinical report on the value of reserpine in 1953. At a symposium arranged by Yonkman at the New York Academy of Sciences in 1954, many independent clinical reports agreed on the value of reserpine as a hypotensive tranquilizing agent for use in psychiatry, hypertension, geriatrics, and gynecology.

Reserpine is a white crystalline compound, insoluble in water, soluble in alcohol and glycols, and photosensitive in solution. It is a complex chemical containing many methoxy groups, an indole nucleus, and a significant -N-C-C-C-C-N- arrangement. It is readily absorbed from the alimentary tract, and distributed throughout the body. Its fate in the body is not known. It disappears from tissues in about six hours.

The characteristic effects of reserpine come on rather slowly, but are quite prolonged. It is available in tablets, containing 0.25 milligrams each. In addition preparations are available of the powdered Rauwolfia root, and of various extracts made from the root. The daily dosage varies from 0.1 to 1 milligram usually given at bedtime. The clinical results, while developing slowly, are marked and significant. Blood pressure is reduced in hypertension, and marked tranquilization is observed. Tranquility occurs without sleepiness, and over-aggressiveness, tension, and emotional instability seem to be relieved. Rauwolfia has been

found to promote well-being among nervous individuals, reducing complaints of dizziness, insomnia, excitability, depression, and constipation. It is particularly useful in these respects in geriatrics. Furthermore it reduces pre-menstrual tension and nymphomania. Outstanding has been the effectiveness of Rauwolfia in anxiety obsessive-compulsive drives, or excessive inhibition and withdrawal, among psychotic patients. Rauwolfia is well tolerated, and may be used with other drugs, such as veratrum. Occasionally Rauwolfia produces nasal congestion, and may cause some increase in appetite. In some emotionally sensitive patients, reserpine may cause depression. This can be serious enough to include suicidal inclination. The use of reserpine should therefore be carefully controlled, and it should be stopped promptly if depression occurs. Quite as serious is evidence of sodium and fluid retention. This may be enough to cause congestive failure (Perera).

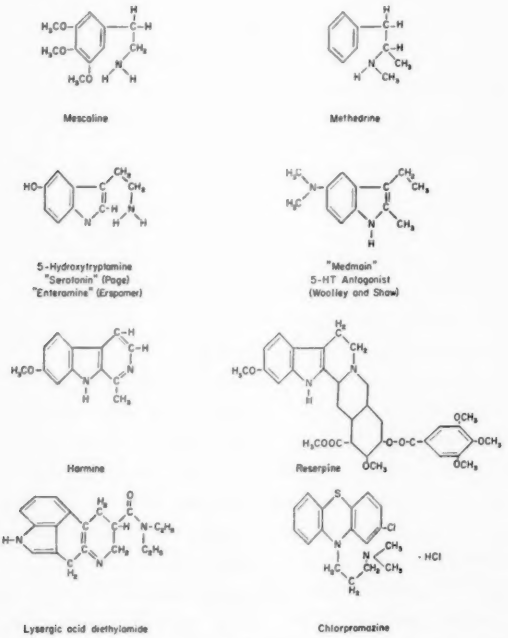


Fig. 2

Chlorpromazine Hydrochloride

Chlorpromazine was developed by the Rhone-Poulenc Specia Laboratories in France, as a possible sedative. This was in extension of the already well-known sedative action of anti-histaminics. Chlorpromazine is 3-dimethyl-amino-propyl-2-chlorphenothiazine hydrochloride. It is a white crystalline powder, fully soluble in water, making relatively stable solutions at a pH of around 5, which remain stable for at least a day. There is some photosensitivity in the product. It is readily absorbed from the alimentary tract and distributed through the body. Its fate is not known, but it appears to be relatively rapidly destroyed or eliminated.

Extensive studies have been made on pharmacology of chlorpromazine. It has relatively low toxicity on single administration, and on repeated administration of the dosage of 10 milligram per kilo orally, the drug gives some depression, decrease in liver weight, and increase in adrenal weight. On long-continued administration no effects are noted on the blood. Chlorpromazine depresses motor activity, abolishes response to a conditioned stimulus, antagonizes drug-induced emesis and motion-sickness. It has no anti-convulsive action, and only a mild adrenolytic effect. As would be expected, chlorpromazine has anti-histaminic action, and also lowers temperature. It relaxes smooth muscle, and potentiates the effect of hypnotics, analgesics and anesthetics. There is some evidence of the development of tolerance to chlorpromazine.

Chlorpromazine has received extensive clinical study abroad and in this country. French investigators noted its central depressants effects and its inhibition of psychic disturbance. The drug was used to produce hypothermia and an artificial hibernation in connection with surgery. Lehman and Hanrahan reported clearly on the use of chlorpromazine as an inhibiting agent for psycho-motor excitement and manic states. Kent and his associates found chlorpromazine useful as an anti-emetic.

Clinical evidence shows the value of chlorpromazine in controlling nausea and vomiting due to a variety of causes including motion-sickness, drugs, radiation and pregnancy. Effective control is obtained by an intra-muscular dose of 25 milligrams three times daily in severe cases, with change to oral doses of 10 milligrams twice daily. For milder cases or for children with viral gastritis, 10 milligrams orally every four to six hours may

be satisfactory. In children 25 milligrams by rectal suppository may be given every four to five hours.

The sedative action of chlorpromazine is marked. It is helpful in controlling psycho-motor agitation and relieves hallucinations and delusions of schizophrenia. More striking is improvement in depressive states. This, however, is often noted only on stopping administration of chlorpromazine. In psychotic patients high doses seem necessary, with an average of around 300 milligrams daily. Dosage should be reduced as soon as improvements are noted. Winkelman finds 25 milligrams three times daily by mouth to be helpful in agitated senile patients. He has also observed marked improvement in the control of anxiety, obsessions, and depression states. Chlorpromazine also may aid in relieving symptoms of Parkinsonism, and it is useful in the control of withdrawal symptoms in addiction to opiates and alcohol.

Chlorpromazine has been used as an adjunct to analgesics in the relief of severe pain in cancer. It is also helpful in nausea induced by radiation, drug therapy, or motion-sickness.

Chlorpromazine has also been used in surgery as an adjunct in premedication and for anesthesia. The use of chlorpromazine preoperatively relieves anxiety, aids to control restlessness in spinal anesthesia, and reduces post-operative nausea and delerium during recovery.

An interesting use of chlorpromazine is in the relief of persistent hiccup. In these cases 25 milligrams is given intravenously. This dosage may be given intramuscularly and repeated in four to six hours.

Response to chlorpromazine therapy is substantial. Usually 80 per cent to 90 per cent of patients treated respond favorably. Unfortunately a number of undesirable side effects occur. The most common is drowsiness, which may ocur in 40 per cent of patients. Hypotensive reactions also occur and care should be used to avoid untoward effect in cardiac disease.

Another unfortunate side effect of chlorpromazine is the occurrence of allergy. This may result from contact sensitization among nurses or physicians. Urticarial rashes may develop in some patients, but clear quickly on withdrawal of treatment. Some patients may show gastro-enteric distress. The most serious untoward effect from chlorpromazine is jaundice. While the incidence is less than 1 per cent, the reaction appears to be due to an infiltration around biliary ducts, with allergic swelling.

Usually the condition clears on withdrawal of the drug. Liver function studies indicate little severe injury. Sometimes a mild fever may occur after a week or so of treatment, with subsequent jaundice.

The administration of chlorpromazine should be discontinued on the appearance of any untoward symptom. If this is done the symptoms will usually promptly disappear, and often the drug may be used again without untoward effect. It is probably wise not to use chlorpromazine in the face of liver disease. Furthermore it should not be used in comatosed conditions due to hypnotics or alcohol.

Meratran and Frenquel

Brown and Werner have found that piperidyl-benzhydrols are interesting agents in regard to action on the central nervous system. a-(2-piperidyl) benzhydrol hydrochloride is called "Meratran," a crystalline bitter compound, rapidly absorbed from the alimentary tract. with distribution through the body, but with unknown fate. With no significant local action, its effects seem to be chiefly upon the central nervous system. Himwich and his colleagues show that the compound stimulates the central reticular substance of the brain, followed by cortical stimulation. It has no effect on appetite, nor does it produce a pressor reaction.

"Meratran" seems to have no significant effects on the autonomic nervous system, and it does not produce wakefulness. It has been used therapeutically in mild depressive states, and is particularly effective in controlling motor tic syndromes. Patients with torticollis are often greatly benefited by treatment with "Meratran," and it may be useful in relieving lumbar muscle spasm in "low-back pain." Clinical reports by Fabing indicate its clinical value in these conditions and show that it has no important untoward side effects, and no significant toxicity. It is administered orally in tablets of 1 milligram each, 3 to 6 times daily.

In studying related compounds Fabing observed that the gamma isomer has remarkably different actions. a-(4-piperidyl) benzhydrol-HCl ("Frenquel") is a white crystalline bitter substance partially soluble in water, and absorbed from the alimentary tract and quickly distributed in the body. Its fate is unknown, but it seems to be destroyed or eliminated in 4 to 6 hours. This compound is not a central nervous stimulant at all, but

rather an antagonist of central stimulation such as may be caused by amphetamine, cocaine, or "Meratran." It potentiates the depressant effect of hypnotic and analgesic agents, and has a very low toxicity on single administration or on repeated dosage.

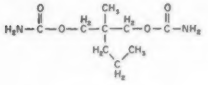
Clinical trials by Fabing and Hawkins show that "Frenquel" brings marked improvement in acute schizophrenic reactions. In chronic schizophrenic patients and in hallucinating seniles, as well as in hallucinating alcoholics, the results have not been as satisfactory, although some improvement is noted. "Frenquel" seems to synergize with chlorpromazine in schizophrenics. On the other hand the drug has little or no effects on psychotic depressive states, nor on obsessive-convulsion disorders. It does not seem to improve anxiety reactions.

In model psychoses produced by lysergic acid diethylamide, or by mescaline, "Frenquel" seems to have an antagonistic effect. If used as a premedicating agent, it will tend to block lysergic acid diethylamide and mescaline psychoses. The name "Frenquel" is given in respect to its ability to quel frenzy. This was analyzed in human experiments in which it was found that the compound can prevent the appearance of model psychoses from lysergic acid diethylamide, or from mescaline. The dosage is 10 mgm. once or twice daily in tablets.

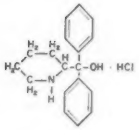
Miltown

During the investigation of a series of propane derivatives, Berger found marked muscle-relaxing and sedative effects from 2-methyl-2-n-propyl-1,3-propanediol dicarbamate. The action of this drug, which is called "Miltown," is similar to that of methanesin, but lasts longer. It antagonizes strychnine and metazole convulsions, and tends to produce a reversible flaccid paralysis of skeletal muscle, without affecting the heart, respiration, or autonomic functions. Its action seems to be on multineural reflexes, but it does not seem to influence mono-synaptic reflexes.

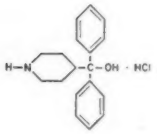
Clinical reports by Selling and Borrus indicate that "Miltown" has marked tranquilizing effects with absence of toxicity. The drug is administered by tablets containing 400 milligrams four times daily. As soon as effects are noted dosage may be reduced and as improvement may continue, the drug may be fully withdrawn. This is usually possible when psychotherapy sessions are systematically held.



"Miltown"
2-methyl-2-n-propyl-1,3-
propanediol dicarbamate



"Meratran"
α-(2-piperidyl) dibenzhydrol



"Frenquel"
α-(4-piperidyl) dibenzhydrol

Fig. 3

"Miltown" is useful in anxiety and tension states. It aids in clearing behavior problems, conversion hysteria and alcoholic withdrawal symptoms. However, it is not so promising in psychoses.

It is concluded that "Miltown" is a practical, safe and clinically useful central nervous system depressant, which is not habit forming, and which has very low toxicity. It is most effective in reducing tension, irritability, and restlessness, and may be helpful in epilepsy and paralysis agitans. It promotes restful sleep and general muscle relaxation and is helpful in neurogenic skin conditions, abdominal discomfort and tension headache.

Lysergic Acid Diethylamide

It is questionable whether or not lysergic acid diethylamide has significant clinical usefulness. Certainly it is of the utmost importance in theoretical analysis of the etiology of neuropsychoses, and is an extremely important experimental tool. Yet its use in healthy human subjects experimentally may be ques-

tioned unless the utmost care is followed to prevent harm. Certainly no responsible physician should run the risk of inducing a psychosis in a susceptible patient through its experimental use without being prepared to handle any possible consequence.

On the other hand, Sanderson and his associates have used the drug in 25 gamma doses orally, at weekly intervals, in "drug-assisted psychotherapy," both for in-patients and out-patients. Careful instructions are given for the organization of the professional team, the character of the medical visits, the role of the nursing staff, the general environment of the treatment session, the possibilities of group therapy, and specific instructions for the preparation of the patient after treatment.

With this careful approach, Sanderson offers evidence that the drug opens the unconscious so that repressed memories are relived with remarkable clarity, with resulting opportunity for the psychotherapist and the patient to obtain insight and understanding. The use of the drug at weekly intervals in these psychoanalytic sessions avoids the possibility of development of tolerance. If the drug is administered daily, there is rapid tolerance so that the effectiveness of the drug is progressively reduced. This tolerance is lost rapidly.

Liddell and his colleagues have studied the clinical action of lysergic acid diethylamide, in comparison with dN-methyl-amphetamine-HCl (Methedrine). The latter was used in dosages of 50 milligrams whereas lysergic acid diethylamide was given in dosages of 50 gamma, both intravenously. Both drugs aggravate the existing clinical picture. Both drugs cause an initial rise in plasma adrenaline, with a secondary fall and a subsequent rise. Lysergic acid diethylamide causes more fluctuations in mood than does methedrine, and euphoria predominates.

Lysergic acid diethylamide has been proposed as a diagnostic agent, in order to uncover the latent tendencies of a patient with respect to depression, or schizophrenia. W. A. Stoll suggested its use as a personality test.

The effects of lysergic acid diethylamide appear within a half hour after administration, and include both autonomic responses of skin-reddening with vascular changes, and central effects of disorientation. Within an hour other autonomic effects may occur, such as chilling. The effects of 50 milligrams of lysergic acid diethylamide by mouth usually last four to five hours. Its effects are often compared to those of mescaline.

Ritalin

Another interesting behavior-altering drug has recently been introduced by Ciba. This is methyl-phenyl-2-piperidine-acetate HCl, called "ritalin" (methyl-phenidylacetate Ciba). It is a white crystalline product, which as the hydrochloride is freely soluble in water and ethanol. A 10 per cent aqueous solution has a pH of 3. It is readily absorbed from the alimentary tract, and appears to be rapidly destroyed in or eliminated from the body.

Methyl-phenidylacetate is a central stimulant. It causes an increase in spontaneous activity in experimental animals in doses of 1 mgm. per kg., with maximum restlessness in about an hour and with gradual recovery without untoward effect in about 2 hours. It has an analeptic effect against short acting barbiturates and counteracts respiratory depression from morphine. It seems to have little effect on smooth muscle, on cardio-vascular function, or on blood. Death with convulsions occurs in experimental animals with single doses around 40 mgm. per kg.

Ferguson has reported on its use in 127 chronic schizophrenic patients of long standing, and covering a wide age distribution. These patients were markedly deteriorated, with strong negativistic response. Of them, 101 showed improvement clinically on administration of 10 to 30 milligrams of the drug, thrice daily by mouth. No untoward effects were noted, and there was no interference with sleep. In some instances improvement was dramatic after several days administration, while in other cases improvement occurred more slowly.

Summary

Fascinating hypotheses regarding the etiology of mental disorder are developing as a result of considerations of the chemistry and pharmacology of drugs related to nor-adrenaline, 5-hydroxytryptamine, and alkaloids containing an indole nucleus. With coordinated effort on a large scale between chemists, pharmacologists and clinicians, it may be possible through investigation of these hypotheses, with chemical agents now available, to make a "break-through" in our ignorance regarding the etiology of mental disturbance.

In a practical way, the development of Rauwolfia alkaloids, particularly reserpine, has brought physicians an opportunity for tranquilizing anxiety tension, and nervous agitation, and for promoting quiet relaxation, without toxicity, or without caus-

ing excessive drowsiness. In chlorpromazine, physicians have a drug which is useful in helping to bring improvement in schizophrenia. This drug has certain allergic side effects, but fortunately these seem to be easily controlled. In "Miltown" and "Frenquel," physicians also have new and interesting drugs for tranquilizing agitated patients and for producing relaxation and calmness. These drugs fortunately seem to be relatively free from toxicity.

Lysergic acid diethylamide may have limited practical usefulness as an adjunct to psycho-analysis, in uncovering the unconscious, and releasing repressed memories, but its use must be carefully controlled by skillfully planned team-work and under the direction of highly competent psychiatrists.

The practical results from new drugs now available for the management of mental disorder give every promise that improvements will occur if chemists, pharmacologists, and clinicians can work vigorously together in obtaining verifiable data on metabolic abnormalities in mental disease and on the biochemorphic relations involved in drugs affecting mood and behavior. Since mental illness is the major health problem confronting humanity, such an expectation deserves every encouragement so that a break-through may be achieved in the understanding and control of such disease.

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THE ART AND SCIENCE OF MEDICINE

WALTER B. MARTIN*

Twenty-three hundred years ago, Hippocrates observed that physicians must not only be prepared to do what is right themselves, but also to make the patient, the attendants and the externals cooperate. This is, perhaps, the first recorded statement of the importance of the art of medicine.

To make the patient cooperate, one must first gain his confidence and this, in turn, requires not only a knowledge of the disease the patient has but, also, an understanding of the patient as an individual and full consideration of the externals around him. Shrewd observation of the externals, and avoidance of witless interference, characterize the Hippocratic school of medicine for 400 years. Hippocrates had at his command little of the science of medicine as we know it today but he made use of natural methods and practiced the art of healing.

Modern medicine may be said to have begun with Harvey's discovery of the circulation of the blood three hundred years ago. The past half century, however, has witnessed the greatest advances in medical knowledge and its application to the relief of human misery and to the prevention and cure of diseases that has ever occurred in the history of the world. The reform of medical education and teaching begun late in the last century has brought all of our medical schools to a high state of scientific efficiency. They are not only graduating more doctors but these doctors are better trained in the basic sciences and in the several branches of medicine. Under our modern methods of medical teaching, medical education does not end for the young doctor upon graduation. To a great extent, he continues the study of medicine throughout life. At least one year of service as an intern is now considered an essential part of his training. The openings for further resident training are eagerly sought by a large majority of young physicians. The hospitals offering these training courses are better equipped and better staffed than ever before. The research programs of medical schools and teaching hospitals are strongly supported and have expanded rapidly.

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* President of the American Medical Association. Address at meeting of Alpha Omega Alpha Honor Medical Society, The University of Texas Medical Branch, Galveston, May 14, 1955.

This has resulted in greatly strengthening the effective teaching in these institutions. It also serves to draw into this orbit adequately equipped students who are desirous and capable of entering investigative medicine. But this is not all. Through extension courses offered by medical schools and teaching hospitals, through innumerable medical society gatherings and medical journals that deal with every aspect of medical activities, the education and training of the physician in the science of medicine continues throughout his professional life.

Medicine is further supported by numerous ancillary or auxiliary groups. Professional nurses of today are more effectively trained in the knowledge and techniques of their profession than ever before. Thousands of technicians are active in the support of medicine and other thousands are being trained in the technical aspects of medicine in all its phases. These groups also are constantly extending their education.

The development of improved means of transportation and better lines of communication have brought the patient and the doctor very much closer together. The physician now is able to see and properly serve many times the number of patients that could have been served by his grandfather.

All of these factors have brought about great changes in the general medical situation. The problem of the acute infectious diseases has diminished almost to the vanishing point. It is interesting to note that considering five diseases only—namely, typhoid fever, diphtheria, pneumonia, the acute diarrheas of childhood and tuberculosis, if the same death rate prevailed today for these diseases as did fifty years ago, 600,000 people who are now living would have died in the past year. The decrease in infant and maternal mortality has been equally striking. The overall effect has been to increase the expectation of life by twenty years during the past half century.

All of this is said, not in praise of our profession, but to point out the paradox which faces medicine today. The more efficiently it operates, the greater are the burdens placed upon it. The more effectively it eradicates disease, the more patients it has to deal with. The greater its contributions to the total health of the nation, the more it is criticized and the more vocal become the self-appointed guardians of the health of the public. While the results of all these great advances of medicine are evident in the betterment of the health of the public and in the increased span

of human life, it can also be measured in the lessening of human suffering and in economic gains brought about by the saving of life and the lengthening of productive years. What all of this has added to the wealth of the nation is incalculable.

The very sweep of this accomplishment has caught the imagination not only of the public but of lay writers, sociologists and workers in the welfare field. Most of these see only the machinery of medicine, its hospitals, its fine schools, its complicated instruments and its powerful curative agents. They argue rather plausibly perhaps that since medicine has done so well, it would do better if there were more doctors, more technicians, more nurses working in better and larger hospitals more widely distributed and more fully equipped with all of the mechanics of medicine. They "put their faith in chariots and mighty men of valor" and fail to understand that all of these physical facilities must be directed by an educated mind and an understanding heart.

What is wrong in this picture? Why does the number of patients being treated in our hospitals continue to increase? Why are doctors' offices more crowded than ever? Why are the people disturbed, anxious and fearful? Perhaps it is because their eyes also are focused on the machinery of medicine and they have lost the concept of medicine as a healing art. A large number of the patients who crowd the doctors' offices do not present problems of organic disease and those with an organic disease are often suffering with a functional condition more important than a relatively minor organic defect. Search for this organic factor may absorb the attention of the physician and the patient alike and the more important functional disorder may be overlooked and ignored. In this approach, the physician is fully supported by the patient who expects the doctor to make full use of all the mechanical contrivances available and to indulge him in all the latest therapeutic procedures.

If there is something wrong, what can be done about it? Is there something lacking in pre-medical education of the prospective physician or in his professional education in medical schools and hospitals? Has health education and pseudo health education carried out so widely by pamphlets, newspapers, magazines, radio and television backfired and created more fear than it has allayed? Has the physician lost the ancient concept of medicine as a healing art? Has the physician himself in his zeal

for and absorption in the science of medicine become more interested in disease than in people?

In American high schools and colleges, increasing emphasis year after year has been placed on the practical. Education has been held forth as a means of gaining economic advantage, or of promoting one's social standing. Premedical courses in college have become crowded by requirements in biology, chemistry, and physics to the exclusion of the broadening cultural subjects. Nowhere in premedical training is emphasis placed upon subjects pertaining to the student's relation to his fellow man. Language, philosophy, history and the social studies are largely neglected.

In the medical school, the students live in a world apart, in a vacuum where he devotes his time almost completely to the subjects related to the science of medicine. He sees and hears much of the diagnosis and treatment of disease but little of the treatment of people. That subject, if considered, is only introduced in a brief course in psychiatry or under the solemn title of psychosomatic medicine. The curricula of medical schools are crowded. New subjects are pressing for attention. Somewhere in the pursuit of this surging science, the human aspect of medicine is lost. During his post graduate training in hospitals, the student may have one year of rotating internship which does serve to give him a broader view of medicine. He is likely, however, to embark on a course of training in some particular specialty and from thereon he develops gun-barrel vision. Early in his career, he may settle on some paritcular sub-division of medicine and never acquire a broad general viewpoint as he becomes absorbed in the details and techniques of the specialty that he has chosen. If this process is continued through his postgraduate period, his perspective is likely to be limited to the field of his particular specialty. In general, the medical student lives in a protected world, protected from the assaults of everyday life, and the experiences of the average citizen. He feels that he is properly concerned with the development of his knowledge of medicine as a science and this leaves him little opportunity to observe medicine in its relationship to an individual human to the body politic or to the general affairs of men. He receives little or no instruction in medicine as an art, or in the basic philosophy of medicine. If he enters a specialty without having engaged in general practice, he may never have the opportunity to acquire

a broad outlook on medicine as a whole or on the patient as a whole.

Some of the fault lies in another direction. People are being taught to glorify the specialist and to demean the qualities and qualifications of the general practitioner. Newspaper and magazine writers and the voices on the air are eager to seize on the unusual and sensational experiences in medicine because of their news value. Self-anointed authorities advise the people in every phase of medicine. Giving advice without responsibility may be profitable, but it may also mean disaster to the recipient of the advice. The careful and conscientious work of thousands of physicians laboring day after day attracts no attention and makes no headlines. As a result, people have acquired the habit of rushing to the specialists, who, in their minds, represent final knowledge of the particular parts of their bodies in which, according to their judgment, their trouble lies.

The physician himself also is often at fault. Trained in the modern school and hospital where emphasis is placed upon the unusual and the "interesting," he acquires the habit of making liberal use of all the mechanics of medicine, rather than first determining what can be done by becoming acquainted with the patient, exploring his personal problems and only applying those special and expensive procedures necessary to prove or disprove a diagnosis that he has arrived at by an intellectual, rather than mechanical, means. This, of course, has led to the wasteful over-use of laboratory procedures, the lengthening of hospital stay and has added greatly to the over all cost of medicine. In this mechanical world, the doctor-patient relationship, of which we so often speak, has been mutilated, if not destroyed. Patients have lost much of their confidence in the physician as a healer and look only to the "God of the machine." The highly qualified physician is not just a man with a good memory and a facile mind, nor one merely skilled in technique. He must be a student of people as well as of medicine. He must be able to relate his knowledge of medicine to his patients needs and to the medical needs of his community. He should be a practitioner of the healing art. His knowledge and his interests must go beyond the field of medicine. He should know his community and how the people who make up that community live. How else can he evaluate his patients' problems? He must be cognizant of the factors that bear on the general health of the community and of the

environmental conditions that influence the health of the individual. He must also be aware of the effect of environment upon medicine and strive to improve that environment. For this reason, he should be active in his local society and on his local hospital staff. The quality of medicine in the community will be conditioned by the environment under which it exists. Furthermore, he should go beyond his community; he should be interested in, and have a knowledge of, the economic and social factors that play upon medicine in his state and nation.

The effect of environment upon medicine goes far beyond the community and reaches into the state and nation. Powerful economic, social and political forces are acting upon medicine today. The strong trend toward federal medicine is evident in the rapid growth of the veterans hospital system and the great increase in the number of dependents of service men being treated in our defense department hospitals. Already the federal government has assumed a large responsibility for the care of thirty million of our people. During the last Congress, over four hundred bills affecting medicine and medical care were considered, and, so far, more than one hundred have been introduced into this congress.

Medical education has become increasingly expensive for both the student and the schools. Many schools are in financial difficulty and strong pressure is being brought to bear for federal aid to medical education. I mention these facts to indicate that the environment of medicine is changing and that we cannot afford to remain ignorant of the possible effect of these changes.

There may be an escape from this unhealthy situation. More attention in college should be paid to purely cultural subjects that would round out the student's general knowledge and relate him better to his fellow man. Medical schools should continue this type of education. Medicine should be taught not merely as a science dealing with a variety of diseases, but a calling devoted to not only the control of diseases but more especially to the reduction of the sum total of human misery. The young physician, when he graduates, should already have acquired an interest in, if not a sound knowledge of, man's social and economic environments and his aspirations and of the various forces that motivate individuals in modern society. This process of education should continue through his training as an intern and as a resident. It would be well if all students who desire to enter special branches

of medicine could interrupt their training for a period of general practice. The medical student and the young physician should have a better knowledge of the forces affecting medicine and that may unfavorably change its environment. He should learn something of the value and necessity of medical organizations and of the part they must have in protecting this environment.

The dignity of general practice should be restored. The people should learn to consult first the general practitioner and attach themselves to him as a family advisor. The general practitioner should accept the responsibility of this high and honorable position. He should recognize his limitation in certain fields of highly specialized techniques and should freely seek the advice of the specialists when needed. He should consider always the best interests of the individual who has entrusted his welfare to him. The physician with the narrowed vision of the highly trained specialist should also recognize his limitations in the broad field of general medicine so that medicine may become more personalized to the benefit of both medicine and the people.

The people should learn that there are no final answers in medicine. The sensation of today may be forgotten tomorrow. Out of conflicts of opinions, through discussions, and by careful experimentation, certain generally accepted truths emerge. They should know that medicine advances slowly and painfully toward a goal that it always seeks but has not yet reached. They should learn again of the great advantages in attaching themselves to a personal physician and of trusting him and relying upon him to guide them through the maze of a scientific wilderness, rather than enter this wilderness without a guide.

Our various agents for disseminating news and information should sense their responsibility also. Rather than placing all of the emphasis on the new and sensational, they should use a part of the powerful weapons that they control to advance the knowledge of the public as to the true and great and sound advances of medicine in all its aspects and in all parts of the country.

EPIDEMIOLOGY OF CANCER*

ELEANOR J. MACDONALD

In November, 1952, a working conference was held in Colorado under the auspices of the Association of Medical Colleges and the Conference of Professors of Preventive Medicine, attended by representatives of seventy-six medical schools in the United States and Canada, to re-examine the teaching of preventive medicine in the light of the changing pattern of medical care and medicine education today. A major question studied by the conference was: What knowledge, skills, methods and techniques should all physicians be competent to apply in order to be effective in the field of preventive medicine? The first to be presented was epidemiology. The composite opinions produced the following excellent definition.

"Epidemiology is the study of all factors and their interrelationships which affect the occurrence and course of health and disease in a population. These factors include the characteristics of the host population; the causative agencies—predisposing, precipitating and perpetuating; and the biological, physical and social environment. The objective is to discover the causes of the disease process and to determine the points in its natural history where interruptions may be accomplished in man's favor.

"Epidemiology is much more than the 'study of epidemics.' It has been repeatedly demonstrated that the principles of epidemiology, although applied originally to years of study of communicable disease, also may be applied to the study of a great variety of other disorders, conditions and situations. A few examples of discoveries about noncommunicable conditions made through the use of epidemiological methods are the role of iodine in the prevention of goiter, of fluorides in the prevention of dental caries and of vitamins in the prevention of specific nutritional diseases.

"The word epidemiology has overlapping usages. In one sense it is the body of knowledge amassed to describe the incidence and prevalence of a disease in time, place and group. This is *descriptive* epidemiology. In another sense, epidemiology is a *method*, an orderly scientific approach to the collection and analysis of data that have to do with disease causation and behavior. Such multiple usage can be compared with the meanings of the word pathology, denoting both the description of morbid changes, and the science of the study of these changes.

"Students need to understand both the epidemiological approach and the kind of knowledge required to determine the epidemiology of a particular disorder. The first comprises the basic principles of epidemiology. The second

* From The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas. Received for publication May 21, 1955.

encompasses the methods used to ferret out the multiple causes of disease. To prevent or modify a disease, information such as the following is required:

Disease agent factors: biologic, nutrient, physical, chemical, mechanical or psychologic conditions which contribute to disease occurrence.

The characteristics of man in this connection: his personality, his habits, and customs; inherent characteristics of age, race, sex, etc.; and defense mechanisms.

The character of the environment: its social, economic, biologic and physical aspects.

"Biostatistics is a special skill and fundamental technique in epidemiological study as it provides methods for analyzing facts about variations in the health status of individuals and groups and for measuring the intensity of association among the factors contributing to such variations. Through the study of biostatistics, a critical attitude is gained toward all scientific data, procedures and observations. The concept of the correct design of experiment and methods of collecting and analyzing data is unfolded and a tool designed to focus attention on biologic variability is acquired."

Epidemiology as applied to cancer has the human population as its universe for study. Because this thought is, in a sense, obvious, the difficulties inherent in developing it scientifically and even factually are often overlooked.

Most of what is known of the frequency of cancer has been gathered from death certificates furnished by physicians or government officials. Although every effort is made to fill these out conscientiously, exogenous influences cause profound fluctuations which appear to be changing trends, and which actually are not.

The introduction of each of the six revisions of the International Lists of Diseases and Causes of Death has improved the classification to the extent that knowledge or awareness has increased in the intervals between such revisions. With each one however, the changes have broken the even continuity of the trends and in some cases have distorted the picture. It has taken three centuries to attain the scientific accuracy of disease classification now enjoyed, imperfect though it be. The possibility of shifting classifications bringing about a rise or fall in certain common causes of death makes some reporters question any effort to compile statistics of couses of death. Of these Professor Major Greenwood said in 1942, "The scientific purist, who will wait for medical statistics until they are nosologically exact is no wiser than Horace's rustic waiting for the river to flow away."

The 1948 revision first applied in 1949 effected several remarkable changes, not the least of which were in the cancer cate-

gory. Separate classifications were given to malignant neoplasms of liver, secondary and unspecified, of the lung and bronchus, unspecified as to whether primary or secondary, of the thoracic organs, secondary, and of secondary and unspecified malignant neoplasms of lymph nodes. In each case, this represented an endeavor to eliminate the certification to a metastatic site of cancers of other or unknown primary organs. If autopsies were performed regularly, much of this problem of exact classification would disappear but only about ten per cent of all deaths come to autopsy.

The epidemiologist working with reported mortality from cancer must evaluate and explain these fluctuations in consideration of all known facts, and move cautiously in attributing increase or decrease to what may be the change brought about by classification alone.

Aging of the population should account for a gradual numerical increase in reported cancer equal in proportion to the increase in susceptibles, that is, to those past 50 or in the age groups in which cancer most commonly is diagnosed. There should be a greater increase than this, however, though also a gradual one, attributable to improvement in facilities and techniques, diagnostic and therapeutic. In the early days of state cancer programs, reported increases in cancer deaths were marked at first in each instance and then the rates leveled to their usual gradual line when the programs became integrated. When adjustments for age were made, the female rates leveled and declined slightly in some states. The emphasis on improving the situation in breast and uterine cancer, as well as their accessibility, should occasion a gradual reduction in total recorded deaths from cancer among females, because the largest proportion of deaths from cancer among them fall in these two groups. This is happening. The intensive drive for self examination of the breast was first reflected in an increase in deaths attributed to this cause. Gradually in many areas these are dropping.

Through the centuries, new discoveries have separated one after another of large categories of diseases from the heart and kidney diseases, and from diabetes and cancer. In the life time of most of us, diabetes has emerged from a confused category and been recognized for what it is. Cancer, too, is emerging gradually and should soon be recognized for what it is.

Since the increase in cancer is based on the death certificates, studies from time to time are made to check the current status of their accuracy. Several years ago the author studied the death records for a given year in Massachusetts to find out if the data upon which extensive biometrical methods were being used, were basically accurate enough to warrant this expenditure of effort. Approximately 15,000 individuals were interviewed to verify the data. The death records were first copied on a special card prepared for the purpose. In addition to the name and address of the individual the death record contains information concerning the cause of death, date of first recognizable symptoms, the operative history, the place of death, and the associate cause of death, the record of an autopsy if one were performed, and the name of the individual who signed the certificate. Comparable information was obtained from the family, every physician employed, hospitals attended and social workers who knew the case.

Verification of the facts showed agreement with the death certificates as to age, sex and nationality. Durations were not reliable. This study showed a large error in exact location of cancer. As to the fact of cancer, reporting was 89 per cent correct. Eleven per cent were recognized only at autopsy and not suspected in life. Five per cent were erroneously called cancer. As to site of cancer, agreement was only 75 per cent correct with the greatest discrepancy occurring in liver, digestive tract, respiratory tract and male genito-urinary tract, due to the practice of certifying a death record as cancer of its metastatic rather than its original site.

In continuing the quest for adequate information upon which to base incidence figures, a registry was begun in Connecticut in 1941 backtracking from January, 1935, all cases known to in- or outpatient, laboratory or radiology departments, in general hospitals representing 95 per cent of the total approved general hospital beds in the state. After locating and checking clinically the untraced and living individuals represented for a twelve-year period comparisons were available on the probable accuracy of the diagnoses of different sites of cancer. As reported in 1948, microscopic proof existed in 75 per cent of all cases. In the case of leukemia, breast and female genital tract, microscopic proof was present in more than 90 per cent of the cases. In lip, buccal cavity and others of the upper respiratory tract, 87 per cent were so proved. Again as in the earlier study in Massachusetts the

least adequate diagnoses were in the stomach with 44.8 per cent, lung, trachea and bronchus with 57.1 per cent, esophagus with 57.7 per cent, and liver, brain and male genital tract with less than 70 per cent each, microscopically proved.

More recently a registry has been constructed in Harris County, Texas. All deaths from all causes for this area were copied for 1948, 1949, and 1950 and were matched with the registry from 1944 on, on the assumption that if treatment had occurred, it would have been in the period preceding the death by five years. There were 947 individuals who died with a diagnosis of cancer who were not known to any of these hospitals. When these were broken down by site, more than ten per cent were called primary lung cancer, 30 per cent stomach, liver and other digestive, 5 per cent fell under brain and 12 per cent fell under no primary discovered. Most of these must be considered highly doubtful diagnoses. Still another facet of fact finding came about by this method of checking death certificates for all causes in Harris County with a functioning cancer registry in the area. Of the total 3,636 probable deaths from cancer, 2,430 or 66.8 per cent were known to the hospitals, 947 or 26.1 per cent were never hospitalized for cancer and 259 or 7.1 per cent were hospitalized until shortly before death and almost certainly died of cancer or its complications, but were certified as having died from other causes than cancer. This would correspond with the eleven per cent figure in Massachusetts in an earlier period.

Reports of increases based on death certification in cancers based on clinical impressions without microscopic proof must be scrutinized and explained especially in the cases of those sites which might be metastatic as well as primary, such as lung, liver, lymph nodes or brain.

Another factor which has accounted for an increase of unknown proportions in reported cancer mortality is the priority given formerly to cancer over all other diseases except accidents. The newest 1948 revision of the list of causes of death has changed this. If cancer was mentioned as having ever been present, until 1948 the death was attributed to this cause. If a physician honestly did not know the cause of death, and so stated, he would receive the certificate back with a request for a better diagnosis. "Question of gastric cancer" used to be frequently given in response to such request when the cachectic patient died within days or hours of first summoning a physician. With

the new classification, a physician may state what he considers the primary cause of death to be and it will be so coded.

The effect of intensive publicity in drives for funds for cancer, poliomyelitis and heart disease is reflected in death certification in the United States. This is measurable because such increases are spasmodic in nature and tend to return to normal proportions when the publicity stops or the emphasis is on other sides of the question beside proportionate mortality.

In reported deaths from cancer, from 1941 to 1942, there was an increase of 3,400 deaths over 1941, in 1943 over 1942 the increase was the same. In 1945, the year of the first intensive nationwide drive for funds for cancer the increase was 4,200. The next year when proportionate mortality or one out of seven dies of cancer was the catchword of the drive, reported deaths jumped 6,300. In 1946, the increase was back to 4,500. In 1947, it jumped again to 7,800, in 1948 it was 7,200, in 1949 it was 9,283 and in 1950 and 1951 it dropped back to the earlier levels, to an increase of 4,400 and 4,800 respectively. It jumped again in 1952 to 7,752.

Until 1944, then, the gradual increase in cancer deaths ran a parallel course with the gradual increase in the population. Since then it has reflected the success of the current publicity on cancer. This has been remarked on by others, especially by those most involved. The epidemiologist views it with some concern and asks himself if improvement in a biological phenomenon like the diagnosis of cancer can be other than gradual and be real in advance of the discovery of the cause or cure. Automatically he asks himself, if since the trend of cancer mortality left its normal course with a sharp change from 1945, and there is such an increase in cancer deaths, why is it not reflected in an overall rising death rate? He observes a steady decline in the death rate in the United States to an all time low of 9.0 per thousand. Where then is the decline in other causes to account for the increase in cancer?

Since the reported increase in cancer deaths is largely among males, deaths among males were arranged in the 17 major categories adjusted to the 1949 revision for purposes of uniformity. From 1940 through 1950 the decline in deaths was greatest in the category "infectious and parasitic disease," where reported deaths were cut from 60,000 in 1940 to 33,000 in 1950. The second spectacular drop was in "diseases of the respiratory system other than cancer" from 58,000 in 1940 to 35,000 in 1950. Diseases of the genito-urinary system dropped from 33,000 to

25,000. Senility and ill-defined causes dropped from 17,000 in 1940 to 12,000 in 1950. The three groups representing diseases of advancing age all increased; neoplasms or cancers, diseases of the nervous system and diseases of the circulatory system.

Since the biggest recorded increase in cancer was in cancer of the respiratory system and since the second biggest drop in deaths occurred in other respiratory diseases, breakdowns within each of these categories were made. Within the other respiratory disease deaths, the big reductions were in the pneumonias and influenzas, probably the reflection of the general use of antibiotics.

The use of antibiotics has another side effect, the volume of which is not fully known. It has separated some respiratory cancer from conditions hitherto considered to be chronic respiratory infectious diseases, since the former would not respond to antibiotics and the infections in most instances would. Such a means of differential diagnosis plus Papanicalaou's testing of sputum and other diagnostic advances would account for some of the apparent numerical increase in respiratory cancer, which would actually mark an improvement in diagnostic determinations of lung cancer present all the time rather than an actual increase.

Breakdowns in cancers of the respiratory tract showed that from 1933 on there had been at first a steady increase becoming more marked with each year. Breakdowns within the group show the increase to be gradual for cancer of the larynx from 1,457 in 1940 to 1,873 in 1952. Cancer of the mediastinum and unspecified of this class increased gradually. Cancer of the trachea, bronchus and lung are grouped in the new classification but were separately considered until then. Cancer of the trachea remained fairly constant through 1948, cancer of the bronchus increased from 1,970 in 1940 to 5,838 in 1948, and cancer of the lung from 5,430 in 1940 to 10,493 in 1948. In 1949 the new international classification of causes of death was used for the first time. Awareness that one of the major errors in classifying cancer deaths was the assignment of cancer of the lung, the liver and lymph nodes as primary causes of death for cancers which originated in other organs and metastasized to the lung, liver or lymph nodes gave rise to new categories generally worded as not stated or not known whether primary or secondary. This was to give the physician signing the certificate a chance to state exactly what the situation was as he saw it at the moment of death.

With this refinement made possible, an interesting change occurred in deaths registered as being from cancer of the lung, trachea and bronchus, and as cancer of the liver. The separation of the group of cancers which could not be classified as certainly primary in the lung, trachea and bronchus caused the category to lose 9,835 in 1949 and to total just under 7,000. Some of this drop is undoubtedly due to unfamiliarity with the new classification by the vital statisticians coding the records. By 1952, however, it seems to be stabilizing with 9,716 lung primary cancers, and 11,866 secondary or unknown whether primary or secondary.

Most reports today combine these categories as either "lung cancer" or "respiratory cancer." If there is reasonable doubt on the part of the man who signed the certificate, and saw the patient, this combination of the known primary lungs with those not certainly primary seems unjustified. Care must be exercised by the epidemiologist to evaluate all purported sharp increases in cancer of sites under the glare of extraordinary popular attention, so that the true state of affairs will not be submerged by a popular and understandable stampede to find a correlation between what may in fact be accidental, simultaneous occurrences, and to take the next step, and attribute to this correlation the property of causation.

Further confirmation of the need to exercise caution in this regard is to be found in the similar correction occurring in cancer of the liver, the second most common site of metastases. When the new classification gave an opportunity of stating whether the death was certainly from cancer of the liver, or from another or unknown site and secondary in the liver, half of those hitherto classified as primary became secondary or not stated, with the differential remaining the same, in 1952 for example: 4,446 primary liver and 4,887 secondary or not stated.

No discouragement is implied by this recital of the need for exercising self-discipline and judgment in evaluating reports on the occurrence of cancer. Far from being deterred, the epidemiologist may agree with Englebreth-Holm (1950) in his comments at the conference on geographical pathology and demography of cancer, that "in order to obtain fully reliable results in research work on a demographical basis, the institution of absolute, uniform international methods of registration would be vital. Likewise, the application of a uniform nomenclature is of

fundamental importance. If we lived to see the fulfillment of these theoretical demands, we could almost at once leave further research in the hands of mechanical calculators.

"In experimental cancer research, we have for many years worked without exact methods, but nobody has doubted the justification of the work for this reason, and we are now approaching though slowly, the application of more accurate means."

Certain thoughts persist in the mind of the epidemiologist who has systematically amassed morbidity data. Some of the commonplaces, if explained, might give some of the information needed. If cancer as a whole is considered, it is more common among females. If cancer of the breast and genital tracts are removed from the total, the picture is reversed. Why is it more common among males in the sites of cancer common to both sexes? Is this true of all types of cancer common to males and females and is the ratio consistent? In Dorn's, Clemmesen's and the author's work, the disproportion is present. It is not consistent for all common sites in any of the series. In the 20,000 microscopically proved cases in the Connecticut series males had 59 per cent more total cancer than females. Yet females had 20 per cent more cancer of the eye, ear and salivary glands, 234 per cent more cancer of the thyroid, 43.2 per cent more cancers of the liver and gallbladder, and 9.8 per cent more of the large intestine excluding rectum, than males.

Males had 493 per cent more cancer of the lip, buccal cavity and other upper respiratory sites, 341 per cent more primary lung, 320 per cent more of the esophagus, 131 per cent more of the stomach, 42 per cent more of the pancreas, 68 per cent more of the small intestine, 41 per cent more of the rectum, 129 per cent more of the urinary tract, 29 per cent more leukemia and 41 per cent more lymphoma.

The greatest disproportion of cancer among males occurs in upper respiratory, lung, esophagus, stomach, pancreas, small intestine, rectum, urinary tract and systemic disease. The greatest disproportion in females over males is in thyroid cancer.

What inhibiting factor is present in females that assures them lesser risk of cancer of the common sites than males? Or conversely, what variables are present in males and not in females to a large enough extent to account for this difference? What differences from other females are there in those who do contract cancer of the sites more common to males? Are they inherent or

environmental? Understanding of the exotic and unusual sites of cancer in their natural environment is being pursued by the best brains in the cancer field in all countries represented in the membership of the geographic pathology and demography of cancer congresses begun in 1950 and meeting regularly.

It is generally agreed that no significant population group is free from cancer. Great variation appears to exist among different population groups in the primary sites of cancer. Verification of this is being attempted by the international group just mentioned. Oberling has cautioned that these impressions must be replaced by data based on facts. Lacassagne's suggestion that primary cancer of the liver in various races, cervical cancer among Jewesses and lung cancer be studied exhaustively, simultaneously in each country represented, has resulted in this being undertaken. He recommended a closer collaboration with veterinary surgeons, inspired by the extraordinary frequency of liver changes in swine reported by Ninard and Hintermann in Morocco, especially in view of their conclusions on the transformation of liver cells into cancer cells. This work is in progress.

It has been suggested by Gilliam that the influence of a very large number of factors in cancer causation could be estimated epidemiologically by taking advantage of the truly enormous differences in race, diet, environment and so forth available in human populations.

He has suggested that the direct method of measuring risk has as its point of departure a population, or group of people, instead of a series of cases. The general demographic characteristics of this population such as number, age, race and sex must be known. It should then be divided into those who do and those who do not have the characteristic under suspicion. The groups are then observed and cancer occurring in them is compared. If equal diagnostic facilities are available and used to the same extent, the true incidence of cancer may be determined in each group and valid comparisons of risk among them may be made.

This is the situation that is being sought in the current work on epidemiology of cancer in Texas. The continuing followup registry, from 1944, of clinical records, including complete autopsy protocols when such exist, in two widely separated counties (El Paso and Travis) is more than the hospitalized and death recorded cases. Laboratories, clinics, dermatologists' offices and pri-

vate physicians' offices are being canvassed to complete the roster of diagnosed cases.

With this as a baseline, it is planned to interview the total populations of these two counties (El Paso and Travis) on many of the questions at present extant on industrial or environmental factors The information thus obtained can be checked after collection with the names in the register and valid clinical data should help to ascertain the situation as it is. It is known from the author's own experience that personal population surveys do not give the true cancer story, that deaths from all causes must be verified to determine the real number of deaths from cancer, and that a hospital population differs from a general population. With each of these counties containing Latin American and Negro as well as other American populations, such factors as diet, environment and racial differences may be compared. In both these areas, specific demographic information concerning the number, age, race, sex, housing conditions and residence by census tract is known from the 1950 census. The death certificates are available. The results of X-ray surveys from the tuberculosis program are available. With all this, salesmanship to the community and total cooperation is the final step. In these counties unanimous support of the medical society has been secured in the cancer follow-up program.

Each of the problems related to human cancer and its etiology must have true incidence as its baseline for sound evaluation. Each attempt to arrive at genuine incidence figures reveals new approaches hitherto unrealized. The task of finding out how much and where has been accepted as the first responsibility of the epidemiologist in the field of cancer. Testing the significance of the multiple variables on the disease and on each other follows naturally when adequate base data are assembled.

Major Greenwood, the eminent epidemiologist mentioned above has said of epidemiologists what might well apply to each of the workers in the field of cancer, that for the existing generation a humbler task than that of explanation is imposed, that of description. "Let it be our merit to have arranged and simplified those descriptions of phenomena which someone who comes after us will need in order to test hypotheses suggested not by after dinner chatter or a hasty perusal of uncritically compiled statistics, but by laborious and quantitatively adequate experiments."

TABLE 1

Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death, Sixth Revision Adopted 1948

Malignant Neoplasm of Respiratory System (160–165)

	Total Respiratory 160–165	Nose, Nasal Cavities, Middle Ear, and Accessory Sinuses 160	Larynx 161	Trachea, Bronchus and Lung Specified as Primary 162	Lung and Bronchus Unspecified as to whether Primary or Secondary 163	Mediastinum 164	Thoracic Organs (Secondary) 165
1940	9,887	344	1,457	7,554		532	
1941	10,624	383	1,453	8,331		457	
1942	11,305	358	1,549	8,936	Com-	462	Com-
1943	12,072	332	1,490	9,758	bined	492	bined
1944	13,015	367	1,567	10,537	through	544	through
1945	14,184	362	1,692	11,572	1948	558	1948
1946	15,591	375	1,746	12,878	with	592	with
1947	17,698	379	1,872	14,888	162	559	164
1948	19,364	394	1,868	16,520		582	
1949	19,755	596	1,884	6,825	9,835	378	237
1950	21,503	645	1,852	7,618	10,695	409	284
1951	22,838	607	1,835	8,438	11,287	346	325
1952	24,745	579	1,873	9,716	11,866	296	415

1940–1948 List numbers 55d, 47a, b, c, d, e, f, adjusted to numbers (160–165)

TABLE 2

Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death, Sixth Revision Adopted 1948

Malignant Neoplasm of Liver and Biliary Passages

Year	Total Liver, Primary (155)	Total Liver, Unspecified Primary or Secondary (156A)
1940	9,747	
1941	9,881	
1942	10,204	Combined
1943	10,972	through
1944	11,177	1948
1945	11,207	with
1946	10,804	155
1947	10,572	
1948	10,598	
1949	4,432	5,570
1950	4,653	5,121
1951	4,753	4,943
1952	4,946	4,881

1940–1948 List numbers 46f
1949–1952 List numbers 155 and 156A

Even more than this is requir[illegible] In a period of competition and mass production, we must w[illegible]k ogether and yet remain individualists with leisurely attitudes of mind. We have to keep minds open to any observation that may even remotely bear on this subject. As one of the team, the epidemiologist, realizing that the answers to the various problems are going to have such an integral effect on every life, feels toward them an intense responsibility and is becoming more and more self-analytical in order to bring his best ability to bear. He has come to realize that the essence of epidemiology is sound thinking. Working from a flexible hypothesis and avoiding the fatal fixed idea, the epidemiologist succeeds by following the example of true science and ponders his results without feeling compelled to rush into print until the full maturity of his analytical reasoning is attained. By working toward that essential objectivity, he may become the coordinating element between the specialists of the several disciplines as they place the fruits of their work upon a common table for group consideration.

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EARLY DIAGNOSIS OF SALMONELLA, SHIGELLA, AND COCCUS INFECTIONS BY LYSIS TEST WITH URINE-FRACTIONS*

EUGENE L. NOWICKI

The interest in lytic factors in serum is as recent as the immunology. Coincidently the studies on antibodies, on their application, and on their theoretical aspect have grown to an independent science and have covered a very large field. During the same period the studies on lytic factors have advanced very little, and the origin, the relation to antibodies, as well as the importance of these lytic factors are still very obscure.

The first experimental studies on lytic factor in serum were performed at the end of the 19th century. In 1889 Behring studied lysins in serum of animals. He differentiated Alpha lysins, which in connection with complement produced lysis of gram negative bacteria, and Beta lysins which he found to be nonspecific. Later Mackie, Finkelstein, and Van Rooyan (1932), described in serum a nonspecific bactericidin, active against gram positive bacteria. Tillet (1937) published his observations on bactericidin in human serum, describing it as being highly active against hemolytic streptococci. Greatest activity of the bactericidin in serum was in acute phase of disease. Bloom and Bake (1948) studied bactericidin against *Bacillus anthracis* from several tissues of various animal species. Electrophoretic and chemical studies indicated a basic polypeptide containing a large amount of lysin. Jacox (1950) reported on nonspecific substance bactericidal for *Bacillus subtilis* which appears in the serum of humans in the acute phase of a variety of diseases. This was reported to require calcium for its activation and was most active at pH values near neutrality. Adler (1953) studied the bactericidins for *Salmonella typhosa*. The "normal" bactericidins appear to be specific for antigenic factors IX and XII, which are characteristic for the surface of S-state *S. typhosa* cells. Second antibody directed against a widely distributed antigen (R-antigen) is an inhibiting antibody for bactericidal sensitization of *S. typhosa* cells. Myrvik and Weiser (1955) worked on bacteri-

* Department of Bacteriology and Parasitology, The University of Texas, Medical Branch, Galveston. Supported by McLaughlin Postdoctoral Fellowship Program. Received for publication July 13, 1955.

cidin active against *Bacillus subtilis*. This has been quantitated in the serum of the rat, rabbit, human, guinea pig, cow, and dog. High values were obtained only in the rat and rabbit. Although the serum of healthy humans contains only small amounts of bactericidin, the levels may increase several times during the acute phase of various illnesses. Electron microscope studies have shown that the lysozyme exerts its lytic effect on the cell wall, whereas bactericidin tends to lyse all components of the cell. Probably a similar lytic factor has been studied in urine of rabbits and humans infected with or immunized against leptospira (Nowicki, 1955). The lytic factor against leptospira was isolated from urine by acetone precipitation and concentrated ten times in saline. Various strains of leptospira were incubated with the urine fractions and examined in dark field microscope. The lysins appear in urine very early, several hours after infection or injection of killed leptospira. They are relatively group-specific as the lysins produced against one species of leptospira are active, in various degree, against several leptospira species, but they are negative for other. As the lysis test with urine fraction is an easy technic and could be applied for early diagnosis, this paper is presenting the results of similar experiments with *Salmonella*, *Shigella*, and *Coccus* group of microorganisms.

Material and Methods. A young culture of heat killed microorganisms was used for intravenous injection of rabbits. The majority of rabbits received just 4 to 6 injections. The urine was then collected and precipitated daily. Some of the rabbits were immunized for a longer period, two to three weeks, in order to follow the appearance of the lytic factor in relation to antibody production. The blood was taken from these rabbits every few days and the antibodies in serum examined. The following microorganisms were used for experiments: *Salmonella typhi*, *S. paratyphi A*, *S. paratyphi B*, *Shigella flexneri*, *Escherichia coli*, *Diplococcus pneumoniae*, *Staphylococcus aureus*.

The urine from injected rabbits was treated as described for early diagnosis of leptospirosis (Nowicki, 1955).

The lysis test was performed in two ways: (1) on Petri dishes with solid medium, (2) in 1 to 2 ml. tubes with liquid medium. The technic on solid medium was the following: the medium was inoculated with bacteria, and several sterile discs, soaked in sterile (by filtration) urine fractions, were placed on the medium. The growth of bacteria was observed the following days.

The areas without growth around the discs were evaluated by comparison. The technic with liquid mediums was as follows: the culture of microorganisms was mixed and 0.3 ml. pipetted in 1 to 2 ml. tubes. The sterile urine fractions, 0.3 ml. into each tube, were added, the tubes shaken and incubated at 37° C. During the following days the turbidity of the liquids in tubes was compared with control tubes (0.3 ml. culture plus 0.3 ml. saline).

Results. *Salmonella typhi*, *S. paratyphi A*, *S. paratyphi B*, and *Escherichia coli* were injected into rabbits and the urine fractions used for cross reactions with the organisms of above mentioned species. Table 1 shows the results. The largest area without growth is marked on the table with + + +. Zero means

TABLE 1

Lysis test with urine fractions—Cross reactions within the Salmonella group and with Escherichia coli

Urine fractions from rabbits injected with:	Microorganisms			
	S. typhi	S.p.A.	S.p.B.	E. coli
S. typhi	+++	++	++	0(+)
S. para A.	++	+++	+++	0(+)
S. para B.	+	++	+++	0(+)
E. coli	0	+	+	+++

Urine fraction concentrated ten times.

the colonies were growing in contact with the disc. The lytic factor for each species of *Salmonella* has a group specificity. The urine fraction from the rabbit injected with *S. typhi* has stronger lytic activity for *S. typhi* organisms than for *S. paratyphi A* and *B*. It has no lytic activity for *Escherichia coli* organisms. The urine fraction from the rabbit injected with *S. paratyphi A* has equal activity for organisms of *S. paratyphi A* as for *S. paratyphi B*, and a little weaker lytic activity for *S. typhi*. A very similar reaction is between urine fraction from *S. paratyphi B* rabbit and organisms of *S. typhi*, *S. paratyphi A*, and *S. paratyphi B*. Both urine fractions have no activity, or a very weak one, for *Escherichia coli*. The evaluation of areas around the discs as three or two crosses is a subjective one. Therefore, the urine fraction *S. paratyphi A* reacts practically in the same way with all species of *Salmonella* organisms. The reaction of urine fraction from *E. coli* rabbit is more specific: the lysis test with *Salmonella* or-

TABLE 2

Lysis test with urine fractions—Cross reactions within the Salmonella group and with Escherichia coli

Urine fractions from rabbits injected with:	Microorganisms			
	S. typhi	S.p.A.	S.p.B.	E. coli
S. typhi	++	0	0	0
S. para A.	0	++	+	0
S. para B.	0	+	++	0
E. coli	0	0	0	++

Urine fraction concentrated five times.

TABLE 3

Lysis test with urine fraction of rabbit injected with Shigella flexneri and microorganisms of Salmonella group and Escherichia coli

Urine fraction from rabbit	Microorganisms				
injected with:	S. typhi	S.p.A.	S.p.B.	E. coli	Sh. flexn.
Sh. flexneri ...	+	+	+	++	+++

TABLE 4

Lysis test with urine fractions of a rabbit immunized against Salmonella and E. coli with Shigella flexneri microorganisms

Urine fractions from rabbit injected with:	Microorganism Shigella flexneri
S. typhi	+
S. para A.	+
S. para B.	+
E. coli	++

ganisms is much weaker or negative, comparing with the reaction with *E. coli* organisms. In order to differentiate the mentioned reactions various dilutions of the urine fractions were prepared. Table 2 shows an analogous set of reactions with the difference that the urine-acetone sediments from 100 ml. urine were redissolved in 20 ml. of saline. Therefore, the concentration of urine fractions was five times, and not 10 times as before. None of the tests showed a lysis area of the size as in the first experiment (Table 1), but the specificity was more expressed:

TABLE 5

Lysis test with urine fractions. Cross reactions within the Coccus group

Urine fractions from rabbit injected with	Microorganisms	
	Diplococcus pneumoniae	Staphylococcus aureus
Diplococcus pneumoniae.....	+++	+++
Staphylococcus aureus	+++	+++

TABLE 6

Lysis test with urine fractions. Cross reactions between Coccus and Salmonella, Shigella, and Escherichia

Urine fractions from rabbit injected with:	Microorganisms					
	D.pn.	St.aur.	S.typhi	S.p.A.	S.p.B.	E.coli
D. pneumoniae .	+++	+++	0	0	0	0
S. aureus	+++	+++	0	0	0	0
S. typhi	0	0	+++	++	++	0(+)
S. para A.	0	0	++	+++	+++	0(+)
S. para B.	0	0	++	+++	+++	0(+)
Sh. flexneri	0	0	+	+	+	++
E. coli	0	0	0	+	+	+++

the urine fraction from *S. typhi* injected rabbit gave the lysis only with *S. typhi* organisms. *S. paratyphi A* and *B* are very closely related and even with higher dilution of urine fractions the complete differentiation is not possible. The next experiment was following the relation between the lysins of *Shigella flexneri* and *Salmonella* group. Tables 3 and 4 demonstrate the results. The relation measured with the lysis test, between *Sh. flexneri* and *E. coli* is closer than between *Sh. flexneri* and *Salmonella* group. The cross reactions represented in tables 3 and 4 show also that the lysins for related microorganisms are little specific. Therefore, the following experiments are dealing with the coccus group and their relation to the *Salmonella*, *Shigella*, and *Escherichia*. Liquid media were used for cultures and the lysis test was performed in tubes by mixing of equal amounts of liquid culture and urine fraction. As control the culture was mixed with saline. The incubation at 37° C. followed. In table 5 are presented the results of the cross reactions within the coccus group. In table 6 are the results of the cross reactions between the coccus

group and *Salmonella, Shigella,* and *Escherichia coli.* There is no cross reaction between both groups. Within the coccus group is the same kind of cross reaction as within the *Salmonella-Shigella* groups.

The elimination of the enzymatic factor in urine after injections of microorganisms was also studied. Immunization against *S. paratyphi A* is given as an example (Fig. I). As the curve

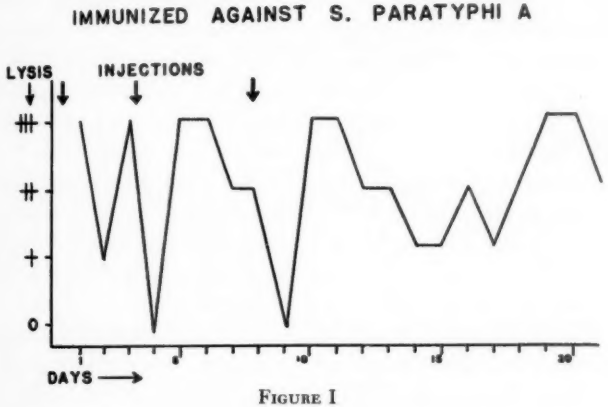

FIGURE I

shows, the lysins appear in urine several hours after the first injection. Every following injection causes a drop of lysins, in general not more than for one day. After the last injection was given, the lysins remain on the level between three and one plus for about 12 days. This picture is very similar to observations described for immunization against leptospira (Nowicki, 1955), as well as against chick ovalbumin (separate study).

Discussion. In comparing the lytic factor in urine (1) during immunization against ovalbumin, as well as (2) against leptospira with the (3) lytic factor for *Salmonella, Shigella, Escherichia coli,* and *Coccus,* the difference is due mainly to the range of specificity. The most specific are the lysins for ovalbumin antigen, then follow lysins for leptospira: they give cross reactions only with some species of the group. The lysins for bacteria investigated in this paper, are the least specific: they give a positive cross reaction within the *Salmonella* group, but further they

react positively with species of bacteria belonging to different groups. Only between entirely different groups as bacteria and coccus, there is no cross reaction. In other aspects the lysins for bacteria and coccus as well as the lysins for leptospira and the enzymatic factor for ovalbumin antigen have many common aspects: (1) in all cases the specificity of lysins (enzymatic factors) differs from the serological specificity, (2) in all cases the lysins are following a very similar curve in course of immunization, no matter what kind of antigen was used, (3) the activity (strength) of lysins or enzymatic factors depends in all cases upon the quantity and frequency of antigen injections, (4) in all cases no direct relation of lysins to antibodies has been noted, (5) the lysins and the lytic factors are probably somehow related to the tissue damage caused by antigen, infection or other factors (as X-irradiation).

From the clinical point of view the lysis test could be useful for early diagnosis of infections in cases when direct bacteriological examinations cannot be performed. It could give an approximate indication of kind of microorganisms involved, what in most cases could be helpful to early treatment.

The further study of the lytic factor in urine may explain its nature, perfect the technic, and open new theoretical, as well as practical applications. Probably the specificity of lytic factors for bacteria and coccus could be increased by using the antigen fractions of microorganisms instead of total bodies.

Summary. The study of lytic factor in urine of rabbits injected with various bacteria and coccus were made. These lytic factors are less specific than the corresponding antibodies. They give cross reactions within the *Salmonella* group and between *Salmonella*, *Shigella*, and *Escherichia coli*. A similar cross reaction was observed within the coccus group. The far related microorganisms as bacteria and coccus do not show cross reactions in lysis test with urine fractions. The elimination of the lytic factor in urine follows a very similar pattern as described for leptospira and ovalbumin antigen. The main difference is in specificity of the various lytic factors, as follows: the most specific is the enzymatic factor for ovalbumin antigen, then the lytic factor for leptospira, and the least specific is the lytic factor for bacteria and coccus.

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STUDIES ON ENZYMATIC FACTOR IN COURSE OF IMMUNIZATION*

EUGENE L. NOWICKI

The interest for enzymatic factors in humans and animals is constantly increasing along with the progress of research on immunity. The appearance of this factor in normal and infected organisms was studied by several investigators. The results, however, of these studies are still confusing. The origin, importance, and even classification of these factors are at present still very obscure.

Some of these factors are active against microorganisms (Tillet, 1937; Jacox, 1950; Myrvik and Weiser, 1955) causing lysis either of parts, or of the whole microorganism. These lysins were observed of humans and animals. Similar lysins were isolated from urine (Nowicki, 1955) during leptospira infection as well as in course of immunization against leptospira.

Another kind of factor with tissue specific enzymatic action was found in urine after X-irradiation of humans and animals (Nowicki, 1942). These factors, specific for irradiated organs (tissues), are probably an expression of abnormal condition of irradiated tissues. A similar organ specific enzymatic factor was also described in urine during typhoid infections (Nowicki, 1943). The nature of these lytic and enzymatic factors was not yet established, they may be similar or very different. At present there is no evidence available to discuss this problem, however they have some characteristics in common: they pass through the normal kidney, appear shortly after irradiation, infection, or immunization, and they disappear after the impulse is eliminated.

The enzymatic factor is of a direct interest to the experiments mentioned below, and, therefore, a short review of the previous results is presented.

It has been observed that 10–16 hours after X-irradiation of a certain organ, an enzymatic factor appears in the urine of irradiated experimental animals. This enzymatic factor is specific for the organ or organs damaged by irradiation. The technic of

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enzymatic reaction, which will be described further, can be used for measuring of intensity of the damage of irradiated organs. This depends upon two factors: sensitivity of the organ (tissue) to irradiation and intensity of irradiation. Also the sensitivity scale of organs, observed by this method, with a very few exceptions, corresponds to the sensitivity scale established by histopathological technic. The appearance of the tissue specific enzymatic factor corresponds to the known phases of tissue reactions after irradiation: the early reaction, the principal reaction, and the late reaction.

A similar enzymatic factor was observed in course of typhoid infection. In a mild form of disease the enzymatic factors were specific for intestinal tract, spleen, and liver. In the high fever stage the specificity of the enzymatic factors was less expressed due to the fact that most of the organs were damaged in some degree by toxins and high temperature. However, the quantitative technic has shown different enzymatic factors which may indicate the coexistence of various organ specific enzymatic factors. In all the cases of infectious diseases, the enzymatic factor was excreted in urine during the course of illness, and could not be demonstrated after recovery. In some cases of clinical recovery the enzymatic factor for intestinal tract was still present. As further course of disease has shown, it was an early sign of a relapse. These observations, if followed by further experiments, may lead to an application of the enzymatic reaction in prognosis.

Materials and Methods. In order to study the enzymatic factor in course of immunization, rabbits were injected with purified chick ovalbumin. Starting with 0.5 mg. of antigen, several rabbits were injected *i.v.*, for three consecutive days, and several rabbits for twenty days increasing gradually the injected ovalbumin to 2.0 mg. per dose. The third group of rabbits was hyperimmunized in order to obtain high active immune serum. Urine was collected daily from all rabbits for forty days and treated as described for leptospira (Nowicki, 1955) with the difference that the acetone sediment was redissolved in a fifth part of urine volume.

The reaction was executed as described under "A," "B," and "C."

A. Preparation of substrate. 1. The crystalline ovalbumin was dissolved in distilled water and coagulated. The coagulate was boiled several times in large amounts of distilled water and left

overnight in refrigerator. The next day it was boiled once or twice more, until the water was clean.

2. One gram of coagulated and rinsed ovalbumin was dispensed into a series of tubes.

3. The tubes with ovalbumin were plugged with cotton and sterilized for 20–30 minutes at 15 lb. pressure.

4. After sterilization the tubes were flamed, plugged with sterile corks and paraffinized.

5. Thus prepared material (ovalbumin) can be kept in refrigerator for several months.

B. Enzymatic reaction. 6. 1 ml. of urine fraction of consecutive days of immunization was added to each tube with sterile coagulated ovalbumin.

7. The contents of each tube was stirred with wooden applicator in order to disperse it uniformly. The tubes were stoppered with corks, and incubated at 37° C. for 14–16 hours.

C. Ninhydrin reaction. 8. After incubation the contents of each tube were stirred again, and 0.5 ml. of the mixture was transferred into 1 ml. tubes.

9. 0.05 ml. of one per cent ninhydrin solution was added to each tube.

10. The tubes were placed in an incubator at 45° C.

11. The progress of the reaction was observed every few minutes through the glass door. When in one or more tubes a blue color appeared, the time in minutes was noted. The minutes were interpreted as reverse related to the intensity of enzymatic activity upon the substrate: the higher enzymatic activity, the more soluble products in liquid, and shorter the time of positive ninhydrin reaction.

In order to study the interference of urine fractions with the antigen-antibody reaction *in vitro*, also the following technic has been tried. A modification of the gel technic was used (Oudin, 1948). The crystalline antigen was dissolved in saline, about 0.5 per cent solution, and five drops of this solution were mixed in several tubes with five drops of urine fractions of the consecutive days of immunization. For these experiments the enzymatic factor from urine was concentrated ten times. The mixtures of antigen and urine fractions were incubated for 16–20 hours at 37° C. A glass plate covered with 1.5 per cent agar was prepared (Fig. I). A long rectangular well was made in the middle of the plate and on both sides of it, eight round wells were

made. A rectangular one was filled with the antiserum of a hyperimmunized rabbit; six round wells with mixtures of antigen with urine fractions, one round well with the antigen diluted 1:1 with saline, and the last one with the urine fraction. If urine contained the enzymatic factor, it would split the antigen and give a different kind of antigen antibody reaction in vitro.

AGAR PLATE FOR PRECIPITATION TEST

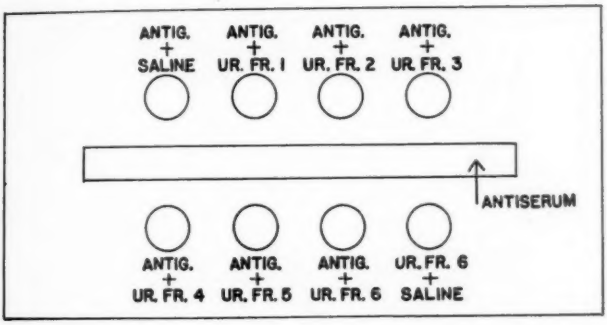

FIGURE I

Results

The purpose of the first group of experiments was to establish the optimal conditions of the enzymatic reaction and the ninhydrin reaction. The set up of these experiments, where only one factor was variable, was as follows:

1) The sediment of the acetone fraction of urine was suspended in different amounts of saline in order to obtain different concentrations of the enzymatic factor. The results are presented in figure II. The intensity of the reaction is expressed in minutes. The concentrations are as follows: I means concentration as in urine, 2x—The acetone precipitate from 100 ml.of urine suspended in 50 ml. saline, 5x—the acetone precipitate from 100 ml. urine suspended in 20 ml. saline and so on. The corresponding curve shows, that the best results were obtained at concentrations 5 to 6 times. The concentration of the enzymatic factor as in urine (mark 1) gave negative results under usual conditions of the reaction. The results obtained with con-

centrations of the enzymatic factor two and three times of urine volume differ considerably from the optimal concentration. The higher concentrations, up to 10 times, are closer to the optimum than the lower concentrations.

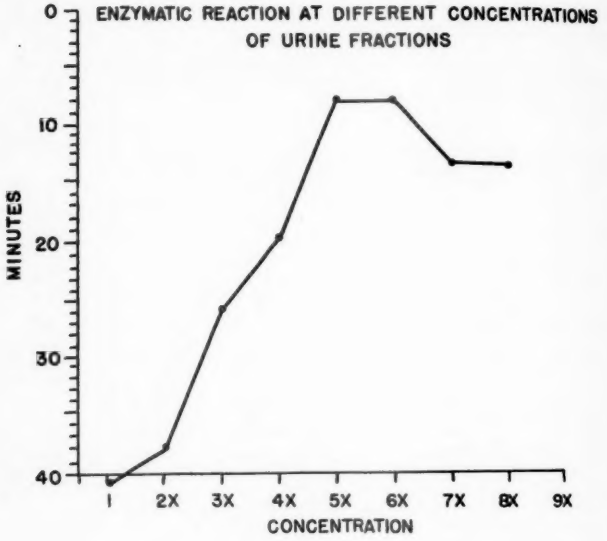

FIGURE II

2) The next curve (Fig. III) presents the changes of temperature of incubation of the substrate and urine fractions. At 37° C. the enzymatic activity was optimal: at 50° C. it was about two times lower, below 20° C. there was practically no activity.

3) Further factor in the experiment was the pH of the liquid during the incubation period (Fig. IV). The phosphate buffer was used. Some of the liquids with various pH were neutralized after the incubation and before the ninhydrin reaction (broken line). The unbroken line shows those liquids which were not neutralized. Both curves are similar on the acid and alkaline side. Both are identical at pH 7. The optimal pH for the enzymatic activity of the urine fraction is between pH 7.4 and 8.0. Toward higher pH the curve is declining rapidly; it was decided therefore, to use the pH 7.5 as being safer because some changes of pH may occur during the incubation period.

4) The next step after incubation was the ninhydrin reaction. Its problem was to establish whether ninhydrin is reacting quantitatively with amino acids, that is whether the time in minutes of the positive reaction (blue coloring) is representing the con-

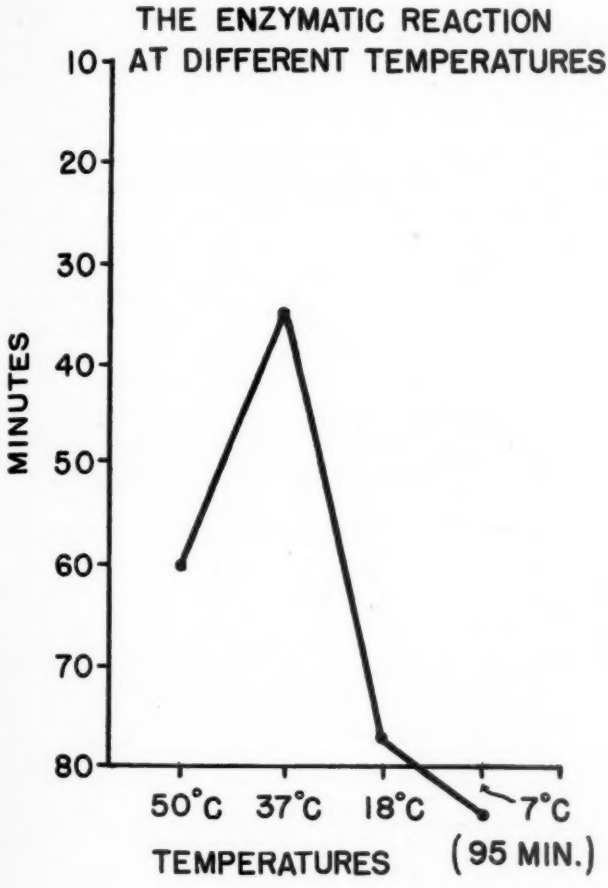

FIGURE III

centration of amino acids. Therefore, serial dilutions of an amino acid were prepared, and the ninhydrin reaction was performed at various temperatures (Fig. V). One per cent solution of glycine

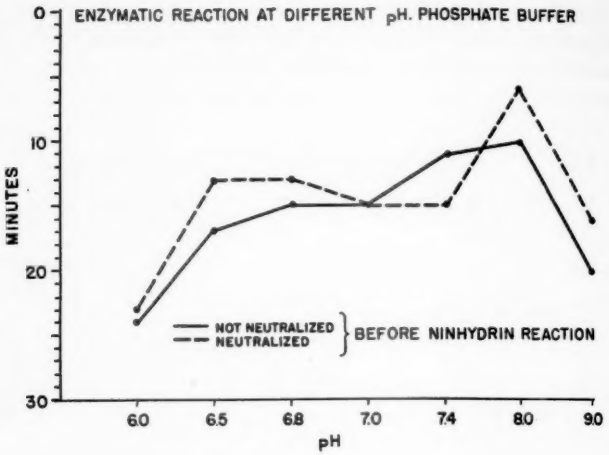

FIGURE IV

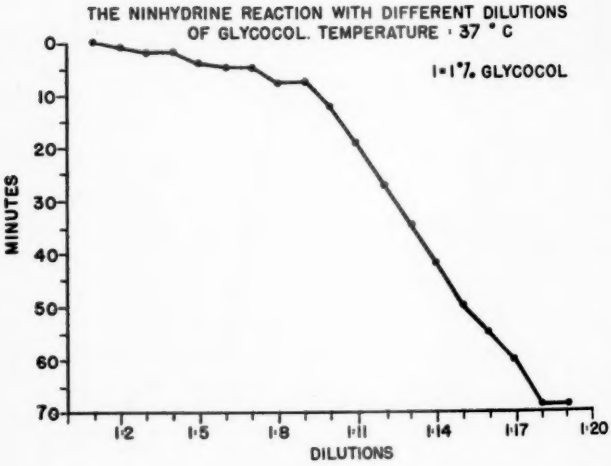

FIGURE V

was used to prepare the dilutions. Each of the dilutions was mixed with ninhydrin in usual proportions. The temperature of incubation was 37° C. The curve presented in figure V shows

that with one per cent glycine solution, the blue color appears immediately. In dilutions 1:2 to 1:8 the line declines slowly, in dilutions 1:11 to 1:18 it declines rapidly. The dilutions 1:18 to 1:20 which correspond to approximately 0.05 per cent glycine solution, gave a negative result. Hence, the reaction at 37° C. is quantitative only for certain range of concentrations. For concentrations outside of this range, the ninhydrin reaction becomes qualitative. In the above case the range of quantitative ninhydrin reaction at 37° C. is between 0.5 and 0.05 per cent glycine. The reaction is qualitative for concentrations higher than 0.5 per cent and lower than 0.05 per cent. Other experiments have shown that, by using various temperatures of ninhydrin reaction, the range of the quantitative results can be changed. For example: at 60° C. the dilutions of glycine between 0.05 per cent and 0.005 per cent gave quantitative results. By lowering the temperature to 20° C., the range of concentrations between 0.5 and 1.5 per cent appear quantitatively. Therefore in using the ninhydrin reaction for experiments or practical purposes, first the temperature 45° C. should be used. In case if several tubes show blue color simultaneously, the reaction may be repeated at 37° C. If the reaction is negative in all tubes after 30 minutes, higher temperature (like 55–65° C.) may be used.

5) In order to see if other amino acids are giving the same reaction with ninhydrin, the experiment presented in table 1 was

TABLE 1

Ninhydrine reaction at 37° C. with amino acids

Min.	Tryptophan 41 mg/10cc	Glycocol 15 mg/10cc	Leucine 26 mg/10cc	Phenylalanine 33 mg/10cc	Thyrosine 36 mg/10cc	Histidine 31 mg/10cc
			A. BORAX BUFFER			
3....						+ blue
25....	(+) light brown		+ purple	+ light brown		
46....			blue	brown		dark blue
65....	brown	0	blue	brown-purp.	0	dark blue
			B. PHOSPHATE BUFFER			
½....		blue				blue-purp.
5....				blue-purp.		
6....			blue			
30....	0	dark blue	blue	blue	0	dark blue

carried out. Various amino acids in corresponding molar concentrations were mixed with ninhydrin solution and kept at 37° C. With borax buffer at pH 6, 0, histidine reacts with ninhydrin as the first giving blue coloring. Tryptophan and phenylalanine gave with ninhydrin a light brown color at the same time as leucine purple one. Glycine and tyrosine do not react in borax buffer. In phosphate buffer at pH 8.0 glycine reacts with blue color very rapidly and histidine at the same time with blue-purple. Phenylalanine reacts with blue-purple color (with borax buffer light brown). Leucine reacts in the similar way. Tryptophan does not react in phosphate buffer, nor does tyrosine. Table 1 shows that only some of the animo acids react with ninhydrin giving blue color; others do not react at all, or give different colors. The latter point of the technic is still confusing, and needs further investigations by biochemical methods.

The second group of experiments dealt with appearance of the enzymatic factor in urine of immunized rabbits. Several procedures of immunization were used in order to establish the relation of the appearance and intensity of the enzymatic factor to dose and fractionation of the antigen. Figure VI represents a

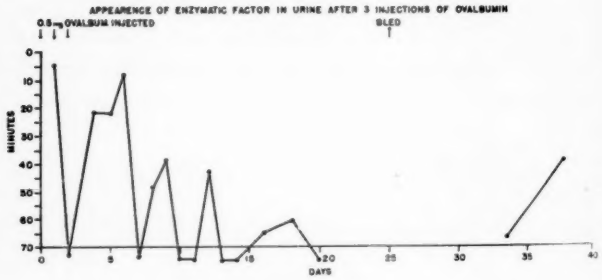

FIGURE VI

simple experiment: three intravenous injections of 0.5 mg. ovalbumin during three consecutive days. The first strong reaction of substrate with urine fraction appears in about 14 hours (earlier urine was not taken). The same day a second injection was given. On the following day the curve dropped to zero. At that time the third injection was given after which the reaction was for three days on a high level and then started to decrease to fall to zero on the twentieth day. The reaction remained nega-

tive till the 34th day when it started gradually again to increase during the next six days. This late reaction following a considerable period of negativity, is very interesting if compared with the analogous late reaction after X-irridation. What has been known as characteristic for the X-ray, seems to be more general tissue response to irritation.

A result of a prolonged immunization is shown in figure VII. For three consecutive days 0.5 mg. per day ovalbumin was injected. After three days of rest, followed injections of 1.0 mg. ovalbumin for four more days. After four day interval, 2.0 mg.

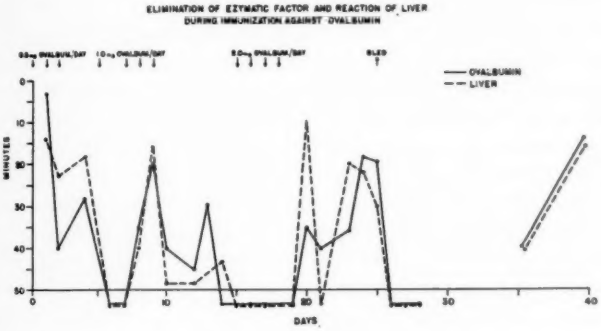

FIGURE VII

daily for four days. The curve differs from the previous one in several points: the reaction is positive for a longer period of time, it is stronger between the tenth and twenty-fifth days and the periods of negative phases are longer. On the other hand there are several similarities: the continuous injections are depressing the enzymatic factor, the maximum of the activity is within a few days after the injection, or as in this case, after a series of injections. The general character of the curve shows first the early reaction, then the principal, and finally the late one. Comparing with the figure VI, it can be noted, that the intensity of the principal reaction between 20th and 25th days, is due to the repeated injections. The time of appearance of the late reaction seems to be independent from the quantity and frequency of the injections. It appears in both figures around the 35th day. But the intensity of the late reaction depends upon quantity and frequency of previous injections (see Figs. VI and VII). This kind

of tissue reaction is already known in radiology (except for very prolonged irradiations).

The urine specimens collected from immunized rabbits were also used in reactions with various rabbit organs, which were previously boiled and sterilized in tubes, 1 gm. in each, as it was described for ovalbumin. Figure VII shows an example of the reaction with rabbit liver. In its general aspect the reaction of the liver specific enzyme is similar to the reaction of the antigen specific enzyme. Nevertheless, some differences are remarkable: (1) the immediate reaction of liver is stronger on the 5th, 9th and 20th days. (2) usually the increased reaction with liver substrate proceeds the peak of the ovalbumin curve, as on the 4th, 22nd, and 23rd days. (3) usually the negative phase of liver reaction (the resting capacity according to the roentgenological terminology) is more expressed: between the 10th and 19th, and on 21st days. The other organs were also investigated. They gave a very similar general picture but they differ in intensity in correspondence with their sensitivity or irritability scale. It could be assumed that the positive reaction with various organs is the result of irritation or hyperactivity of these organs caused by immunization. The question whether the enzymatic factor against antigen (ovalbumin) is related to the abnormal condition of organs, cannot be answered now, but it is an interesting working hypothesis for the future.

The next question was the specificity of the enzymatic factor for antigen. Table 2 shows the result of one of the corresponding

TABLE 2

Urine fractions from rabbit immunized against chick ovalbumin
Cross reactions with other ovalbumin and with ovoglobulin

Min.	Ovalbumin		Ovoglobulin	
	chick	duck	chick	duck
19	+			
44	+++	+		
100	+++	+++	—	—

experiments. The urine fraction from rabbit immunized against chick ovalbumin was incubated with duck ovalbumin as well as with chick and duck ovoglobulin. The reaction has shown a group specificity within the ovalbumins. Nevertheless, the dif-

ferentiation is possible. As table 2 shows, the ninhydrin reaction with duck ovalbumin appears 25 minutes later than with chick ovalbumin. Between the enzymatic factor for chick ovalbumin and both ovoglobulin substrates (chick and duck) there is no cross reaction. It proves that the duck ovalbumin is closer related to chick ovalbumin than the chick ovoglobulin. This is a well known serological fact and is in this connection of interest from the point of view of evaluation of specificity of the enzymatic factor in urine.

The third group of experiments was dealing with interference of the enzymatic factor with the antigen-antibody reaction *in vitro*. If the enzymatic factor for ovalbumin really possesses a splitting activity upon the antigen, it should change the precipitation reaction between antigen and the specific antiserum. For this purpose the antigen was mixed with the urine fractions from immunized rabbit and kept for several hours at 37° C. Then the mixtures were used for the antigen-antibody reaction, as shown in figure I.

Figure VIII shows one of the corresponding experiments. The rectangular well, in the center of the plate, was filled with anti ovalbumin serum from hyperimmunized rabbit. The round well No. 1 with antigen, diluted two times its volume with saline. This gave a normal precipitation line with antiserum. Well No. 2 contained the antigen after incubation with the urine fraction from the first day of immunization (ninhydrin reaction—5 minutes as in Fig. VI). There was no precipitation line. Well No. 3 had the antigen after incubation with the 6th day urine fraction (8 min.—Fig. VI) no precipitation line appeared. Well No. 4 had antigen incubated with the 8th day urine fraction (45 min.—Fig. VI). The precipitation reaction appeared in two lines. Well No. 5 had antigen with the 7th day urine fraction (more than 70 min. in Fig. VI, evaluated as below zero point). The precipitation line is identical with the reaction of well No. 1. The wells No. 6 and No. 7 had the urine fraction (from another rabbit than in Fig. VI) of 20 and 25 min. notation. This gave a very weak double precipitation line. No. 8 had the first day urine fraction without antigen. There was no reaction. If comparing the reactions between the antiserum and liquids in wells 1–8, a conclusion can be made that a strong reacting enzymatic factor, as in wells 2 and 3, decomposes the antigen in the way that it does not give the specific precipitation reaction with the corresponding

antiserum. Enzymatic factor of a medium strength, as in well No. 4, decomposes the antigen in the way that it gives a specific reaction with antiserum in the form of a double line as if there were two specific antigens with different diffusion capacity. In order to support the above theoretical interpretation, the following experiment was performed: the antigen (ovalbumin) was mixed in a series of tubes with different dilutions of trypsin (reagent grade) incubated for 16 hours at 37° C. and used for the antigen

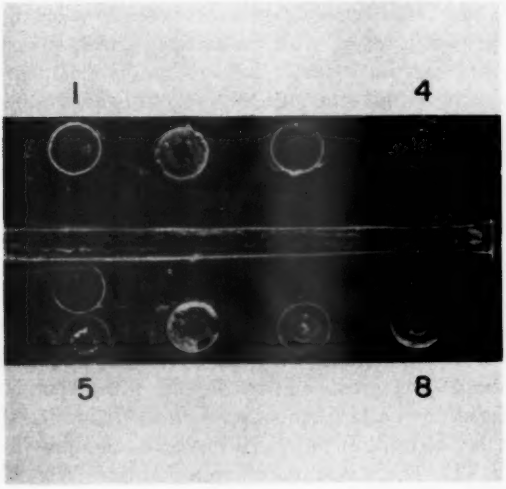

FIGURE VIII

antibody reaction *in vitro*. The results are presented in Fig. IX. Well No. 8 contained the antigen without trypsin. The precipitation line is narrow, white, and with thin ends. Well No. 7 was filled with antigen incubated with high dilution of trypsin (0.05 per cent solution). The precipitation line is double, and more transparent than in well No. 8. The wells No. 6 and No. 5 had higher concentrations of trypsin (0.1 and 0.075 per cent). The corresponding lines are still double but more transparent. The antigen incubated with 0.5, 0.3, and 0.2 per cent trypsin (wells 1, 2, and 3) gave a short, wide, and transparent precipitation lines with thick ends. The incubation of antigen with 0.75 and 1.0 per cent trypsin solution (not shown in Fig. IX) do not give any precipitation lines at all. These results support the above in-

terpretation of precipitation lines after incubation of antigen with urine fractions.

In order to examine the specificity of the enzymatic action upon the antigen, various urine fractions from rabbits, which were in other experiments, were used for the precipitation test

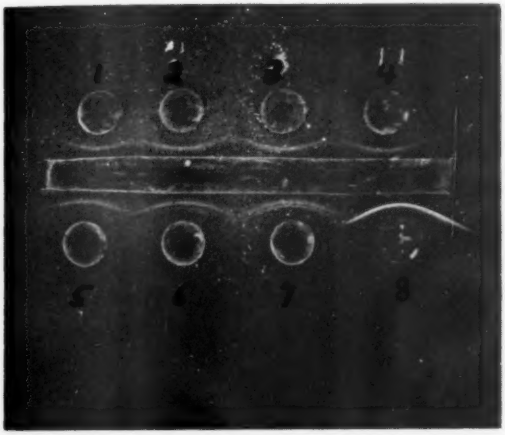

FIGURE IX

in vitro. Figure X represents the results. The rectangular well was filled with anti-ovalbumin serum, the round wells with ovalbumin incubated previously with urine fractions from rabbits immunized against: Well No. 1—*Salmonella typhi;* Well No. 2—*S. paratyphi A;* Well No. 3—*S. paratyphi B;* Well No. 4—*Shigella flexneri;* Well No. 5—*Escherichia coli;* Well No. 6—*Staphylococcus aureus;* Well No. 7—*Diplococcus pneumoniae;* Well No. 8—ovalbumin without urine fraction, diluted 1:1 in saline; Well No. 9—ovalbumin incubated previously with urine fraction from a patient with amebic liver abscess; Well No. 10—ovalbumin incubated previously with urine fractions from a patient with lung tuberculosis. All precipation lines between antiserum and antigen treated with nonspecific enzymatic factors are identical. The above mentioned urine fractions were active against specific substrates, but they had no activity upon the ovalbumin. The urine fraction from rabbit immunized against ovalbumin had no action upon substrates as bovine serum

globulin, whether upon above mentioned microorganisms, or upon human organs.

Discussion. The first part of experiments, dealing with conditions of incubation of the substrate with urine fractions as well as with ninhydrin reaction has shown the flexibility of the method. Some of the factors as pH during incubation, concentration of the urine fraction, relation between substrate and urine fraction, time of incubation and others, have to be at the constant,

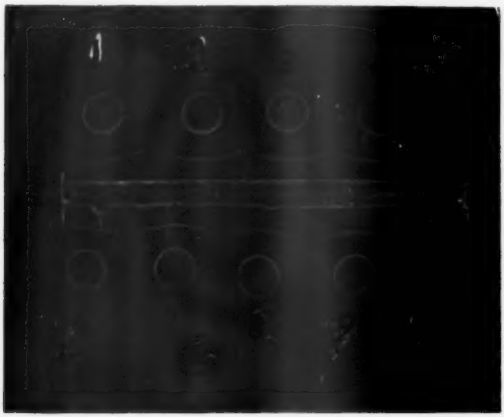

FIGURE X

optimal point, in order to receive the best results. Some factors as temperature and pH of the ninhydrin reaction may be variable. Change of temperature of ninhydrin reaction permits the quantitative evaluation of different concentration ranges of incubation products. Therefore, the temperature of the ninhydrin reaction should be established experimentally for every new problem. The optimal pH for the quantitative ninhydrin reaction cannot as yet be fixed. Several experiments are still required in order to establish the variety and the relation of the products which appear in solution after the incubation period. This point is the weakest one in evaluation of the results, and probably the majority of errors is due to it. Nevertheless, the presence of an enzymatic factor as early response to immunization seems to be close to reality. The interesting point of the problem is the relation of the enzymatic factor to the antibody formation. After several

injections of antigen, as shown in figure VI, the enzymatic factor disappears while the antibody begins to appear, except the late reaction around the 35th to 40th days. On the other hand, during hyperimmunization the enzymatic factor remains as long as the antigen is injected. In this case it appears together with antibody for considerable period of time. Even in the hyperimmunization the enzymatic factor appears in late reaction, independently from the antigen introduction. These facts are an indication for the hypothesis, that the enzymatic factor is not, or very little, related to the antibody production and indirectly related to the presence of antigen. Especially the late reaction speaks for the direct relation of the enzymatic factor to the condition of tissues (organs). The introduction of antigen produces some abnormal condition of tissues as an immediate response as well as late reaction. The enzymatic factor is appearing at the same time as the abnormal tissue conditions, and it is probably in some way related to the latter.

An analogous phenomenon, as mentioned before, was observed as result of the X-irradiation. It is even within the limits of possibility that some phenomena connected with infectious diseases could be interpreted as a late reaction of tissue. An example on typhoid: After clinical recovery and feverless period, some patients develop intestinal perforations without having any other symptoms of relapse. Only the enzymatic reaction has shown a rapid increase of enzyme specific for intestinal substrate, one or two days before perforation occurred. In these cases the intestinal tract, as the most affected during the disease, may develop a strong late reaction which in some patients manifests as perforation. A logic conclusion is supporting this hypothesis: if the tissue develops the late reaction after X-irradiation, as well as after antigen introduction, why should it not react in the same way after infection.

Summary. The experiments of this work are dealing with three problems: (1) the technical conditions of the enzymatic activity of the urine fraction of immunized rabbits upon the antigen, as well as ninhydrin reaction, which was used for quantitative detection of products of the enzymatic action. (2) The appearance of enzymatic factor specific for the antigen in course of immunization. (3) The interfering action of the antigen specific enzymatic factor in urine with the antigen-antibody reaction *in vitro*. (Ad 1): The optimal conditions of the reaction were established

concerning the pH, concentration of urine fraction and amount of substrate used for incubation. The temperature factor of the ninhydrin reaction was considered as variable, depending on the range of concentration of products of the enzymatic activity which is investigated. Therefore, for every problem, the optimal temperature should be established by experiment. (Ad 2) During immunization the antigen specific enzymatic factor appears in urine shortly after the first injection of antigen and remains until the immunization has been completed. After a few injections of antigen, the enzymatic factor disappears before the antibodies have reached high levels. During a long period of injections, as in hyperimmunization, the enzymatic factor appears together with strong antibodies, until the antigen is given. In both cases the enzymatic factor appears again many days after the immunization has been completed. This late appearance of enzymatic factor is probably related to the reaction of tissues affected during the immunization. In discussion this late reaction is compared to the analogous reaction of tissues after X-irradiation. (Ad 3) In the third part of experiments, it has been demonstrated by gel technic that the enzymatic factor in urine is interfering with the antigen antibody reaction *in vitro*. This interference is specific and manifests itself in two ways: a strong enzymatic factor decomposes the antigen in the way that the reaction becomes negative. The weaker enzymatic factor splits the antigen partially and manifests its activity in a double line precipitation.

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FURTHER STUDIES ON THE EFFECT OF SIMULATED ALTITUDE ON EIGHT ADDITIONAL SPECIES OF ARTHROPODS*

ARDZROONY PACKCHANIAN AND MARY PINKERTON

The transportation of exogenous arthropods and their eggs accidentally lodged upon the surfaces of aircraft is an important public health and military problem. Several investigators have collected live insects and insect eggs from the surface of aircraft on short flights at low altitudes: Aziz, 1948; Carneiro de Mendonça, 1947; Hughes, 1949; Griffitts, 1931; McFarland, 1953.

The effects of various simulated altitudes on five species of insects have been reported by Packchanian (1953, 1954). The present communication deals with the altitude tolerance of eight additional species of normal and infected arthropods.

Materials and Methods

Altitude Chamber (Low Temperature Refrigerator with Low Pressure Chamber). The apparatus used in this study was designed and has been described by Packchanian (1953, 1954). This equipment made it possible to reproduce various low temperatures and reduced atmospheric pressures which occur in the atmosphere at high altitudes. The use of hygrometers and several temperature and pressure recording instruments and the manner and speed with which pressure was reduced and normal pressure restored in the chamber have been described in detail in a previous communication (Packchanian, 1953). Additions to the equipment include a portable recording thermometer placed in the altitude chamber, and an indoor-outdoor thermometer mounted on the outside of the chamber door with a bulb inside the chamber connected to the instrument by a capillary hermetically sealed through the door as an additional check of the temperatures.

All of the tests were performed in Galveston, Texas, at sea level, during a period in which the barometric pressure ranged from 755 to 775 mm. Hg.

Arthropods. The arthropods used in these studies included: *Amblyomma americanum*, a tick (vector of Rocky Mountain spotted fever); *Anopheles quadrimaculatus*, a mosquito (vector of human malaria); *Apis mellifera*, the honey bee; *Drosophila melanogaster*, the fruit fly; *Oncopeltus fasciatus*, the milkweed bug; *Rhodnius prolixus* and *Triatoma phyllosoma*, blood suckers

* From the Department of Bacteriology and Parasitology and Laboratory of Microbiology, School of Medicine, The University of Texas, Galveston. The writers express their thanks to Frank Pindak and Jack Stevens, of this laboratory, for much valuable assistance and interest in this study. Thanks also are due to Dr. W. C. McDuffie of Orlando, Florida, for a liberal supply of the eggs of *Anopheles quadrimaculatus* and larvae of *Amblyomma americanum*. Received for publication May 20, 1955.

(vectors of Chagas' disease); and *Xenopsylla cheopis*, an Oriental flea (vector of typhus, plague, etc.). The above species were reared in the laboratory. Methods used in rearing all of these species of arthropods are essentially the same as described in the entomological literature (Peterson, 1953). Both nymphs and adults of *Oncopeltus*, *Rhodnius*, and *Triatoma* were used in these studies; all *Amblyomma* used were nymphs. Adults only of the other species were used for these experiments.

Methods Used in Determining the Effect of Simulated Altitude (Low Temperature with Corresponding Reduced Pressure) on the Viability of Arthropods. For each test, from 3 to 134 arthropods were used. These were placed in glass jars (covered with gauze) or in test tubes (plugged with cotton). The bees were exposed in small wooden boxes with screen tops. All containers were large enough for the arthropods to carry on their normal activities. Whenever feasible, the arthropods were transferred from the original containers and placed in containers precooled to the test temperatures, immediately before pressure reduction was initiated. Following a definite period of exposure to a given simulated altitude (low temperature with corresponding reduced pressure) in the chamber, the containers were removed quickly from the chamber and the arthropods were transferred immediately to similar but warmer (25° C.) containers or petri plates and kept at room temperatures. Observations of their condition were made within one hour and subsequently at frequent intervals for about two weeks before the arthropods were discarded. Paralyzed arthropods were kept under observation until their death, often for several months.

The Effect of Reduced Pressure at Room Temperature on the Viability of Insects. Four species of insects (*O. fasciatus*, *T. gerstaeckeri*, *T. phyllosoma*, and *R. prolixus*) were placed in vacuum jars and maintained at room temperature for 48 hours under reduced pressure (225 mm. Hg. absolute). At the end of this period, normal pressure was restored and the insects were observed immediately and at various intervals for one week.

The Effect of Low Temperature on T. gerstaeckeri. Several groups of *T. gerstaeckeri* were placed in the altitude chamber and maintained at a temperature of —15° C. at normal atmospheric pressure (760 mm. Hg.). At the end of periods of 15, 30, and 60 minutes, the insects were removed to warm petri plates (25° C.) and observed.

Methods Used in Determining Altitude Tolerance of Micro-organisms in Experimentally Infected Insects. (a) Laboratory-reared normal *Triatoma gerstaeckeri* were fed on a dog whose blood was swarming with *Dirofilaria immitis*; (b) laboratory-reared *Musca domestica* infected with *Herpetomonas musca domestica* were also used in these studies; (c) *T. gerstaeckeri* were fed on a mouse (*Mus musculus*) heavily infected with *Trypanosoma cruzi*; (d) *Rhodnius prolixus* and *T. gerstaeckeri* were likewise fed on mice (*Mus musculus*) whose blood was swarming with *Tr. duttoni*; (e) batches of normal *R. prolixus*, *T. gerstaeckeri*, and *Xenopsylla cheopis* were fed on a rat (*Rattus norvegicus*) whose blood contained many *Tr. lewisi*.

All insects mentioned above engorged readily with infected blood. Within 1 to 9 hours after feeding, in most instances, they were placed in precooled containers and exposed in the altitude chamber at —44° C. at atmospheric pressure of 225 mm. Hg. for one to as long as 24 hours. The thawed insects

were dissected, usually about an hour after exposure and at various intervals within 24 hours, and their intestinal contents were examined microscopically for evidence of viable or dead microfilariae or hemoflagellates. The survival of the micro-organisms in unexposed control insects was followed.

Experimental Data

The experimental data can be summarized as follows:

1. *The Effects of Cold Temperature and Reduced Pressure (Simulating High Altitude Conditions) on Eight Species of Normal Arthropods.* The results of this study are presented in tables 1 to 6. A careful analysis of these data reveals the following facts:

(a) *Amblyomma americanum* nymphs exposed to a temperature of −5° C. at absolute pressure of 522 mm. Hg. (conditions corresponding to 10,000 feet or 3,048 meters altitude) for 24 hours, or to −10° C. at a pressure of 472 mm. Hg. (conditions corresponding to 12,500 feet or 3,810 meters altitude) for two hours, remained viable and resumed their normal activities.

At temperatures of −15° C. with absolute pressure of 429 mm. Hg. (conditions corresponding to 15,000 feet or 4,570 meters altitude), 32 per cent of 41 ticks exposed for 15 minutes were killed, and 39 per cent were permanently paralyzed; increasing the time to one hour under the same conditions of exposure caused death or permanent paralysis in 100 per cent of the arthropods.

At −20° C. with 390 mm. Hg. absolute pressure (simulated altitude of 17,500 feet or 5,340 meters), of 18 *Amblyomma* exposed for 15 minutes, 4 were dead, 2 paralyzed, and 12 apparently unharmed. When the exposure time was extended to 30 minutes, 16 were dead and 8 paralyzed of 30 arthropods used; exposures of one and two hours killed all ticks under these conditions.

Twenty ticks were found dead out of 29 exposed for 15 minutes at −25° C. with 349 mm. Hg. absolute pressure; all specimens exposed for longer times at these conditions were killed. At higher simulated altitudes (−30° C. and −44° C. with corresponding reduced pressures), death invariably resulted after 15 minutes of exposure (see Table 1).

(b) *Anopheles quadrimaculatus* exposed to −10° C. with 472 mm. Hg. absolute pressure were undamaged at the end of two hours. However, at −15° C. with absolute pressure of 429 mm. Hg. (conditions corresponding to 15,000 feet altitude), 30 minutes of exposure resulted in 100 per cent fatality.

(c) *Apis mellifera* exposed for two hours in the altitude chamber at −5° C. with absolute pressure of 522 mm. Hg. were all paralyzed; when this condition was extended to 24 hours, all of the bees were found dead.

TABLE 1

The Effects of Temperature, Pressure and Time on Amblyomma americanum

Conditions in Altitude Chamber						Results	
Temp. °C.	Absolute Pressure mm. Hg.	Corrected Rel. Hum. Per Cent	Total Exposure Hrs.	Min.	No. Ticks Exposed	No. Undamaged	No. Paralyzed	No. Dead
— 5	522	88	4	.	18	18	0	0
— 5	522	88	24	.	10	10	0	0
—10	472	82	.	15	9	9	0	0
—10	472	87	.	30	6	6	0	0
—10	472	78	.	30	7	7	0	0
—10	472	78	1	.	10	10	0	0
—10	472	78	2	.	9	9	0	0
—15	429	83	.	15	18	9	3	6
—15	429	80	.	15	23	3	13	7
—15	429	83	.	30	28	18	5	5
—15	429	77	1	.	12	0	5	7
—15	429	78	2	.	8	0	4	4
—20	390	71	.	15	18	12	2	4
—20	390	76	.	30	18	6	4	8
—20	390	71	.	30	12	0	4	8
—20	390	65	1	.	12	0	0	12
—20	390	65	2	.	12	0	0	12
—25	349	66	.	15	29	9	0	20
—25	349	66	.	30	22	0	0	22
—25	349	66	1	.	10	0	0	10
—30	310	69	.	15	8	0	0	8
—30	310	64	.	30	8	0	0	8
—30	310	70	2	.	10	0	0	10
—44	225	54	.	15	11	0	0	11
—44	225	54	.	30	11	0	0	11

At −10° C. with pressure of 472 mm. Hg., exposure for periods of 30 minutes resulted in paralysis to about 81 per cent, and death to 17 per cent of 36 bees tested; exposures of one to two hours invariably killed all.

Simulated altitude of 15,000 feet (−15° C., 429 mm. Hg.) paralyzed 4 and killed 2 of 6 *Apis* exposed for 15 minutes. Thirty minutes of exposure to these same conditions resulted in death to 92 per cent of 25 bees tested, with no cases of paralysis noted;

those exposed to these same conditions for one hour or longer were invariably found dead.

Four bees were found dead and 9 were paralyzed, out of 13 exposed for 15 minutes at −20° C. with 390 mm. Hg. absolute pressure; at the end of 30 minutes exposure, all exposed bees were killed. Conditions simulating altitudes of 20,000 feet and higher killed all the bees in tests of 15 minutes or longer (see Table 2).

TABLE 2

The Effects of Temperature, Pressure and Time on Apis mellifera

Conditions in Altitude Chamber					Results		
Temp. °C.	Absolute Pressure mm. Hg.	Corrected Rel. Hum. Per Cent	Total Exposure Hrs.	Min.	No. Bees Exposed	No. Undamaged	No. Paralyzed	No. Dead
— 5	522	89	2	.	9	0	9	0
— 5	522	87	4	.	9	0	6	3
— 5	522	93	24	.	6	0	0	6
—10	472	82	.	10	14	14	0	0
—10	472	82	.	15	6	5	1	0
—10	472	82	.	30	36	1	29	6
—10	472	82	1	.	6	0	0	6
—10	472	82	2	.	6	0	0	6
—15	429	77	.	15	3	0	1	2
—15	429	67	.	15	3	0	3	0
—15	429	82	.	30	25	2	0	23
—15	429	78	1	.	15	0	0	15
—15	429	78	2	.	18	0	0	18
—20	390	71	.	15	13	0	9	4
—20	390	71	.	30	19	0	0	19
—25	349	64	.	15	10	0	0	10
—30	310	70	.	15	18	0	0	18
—30	310	67	.	30	18	0	0	18
—30	310	71	2	.	18	0	0	18
—44	225	54	.	15	10	0	0	10
—44	225	54	.	30	10	0	0	10

(d) *Drosophila melanogaster* exposed to −10° C. with absolute pressure of 472 mm. Hg. for 15 minutes were apparently unharmed; when these conditions were extended to one hour, all were paralyzed.

Exposure of *Drosophila* to −15° C. at 429 mm. Hg. for 15 minutes produced paralysis in 58 per cent and death in 31 per cent, while 11 per cent were seemingly unharmed. At −15° C. at the

end of 30 minutes, 36 per cent were paralyzed and the death rate rose to 63 per cent. Further increase of the death rate to 71 per cent was noted when the exposure period was lengthened to one hour. When these simulated altitude conditions were extended to two hours, the mortality rate was 100 per cent.

Exposures of *Drosophila* at −20° C. with 390 mm. Hg. absolute pressure produced paralysis or death in all insects; the percentage of insects killed rose from 9 per cent after 15 minutes to 93 per cent after two hours of exposure.

At −25° C. with 349 mm. Hg., exposure for 30 minutes produced 100 per cent mortality. Similar results were obtained when flies were exposed to −30° C. and to −44° C. for 15 minutes (see Table 3).

(e) *Oncopeltus fasciatus* nymphs exposed to −5° C. with 522 mm. Hg. pressure for four hours apparently suffered no ill effects. After 24 hours at the same temperature, 7 were dead and 6 were apparently unharmed, of 13 insects tested.

Oncopeltus nymphs and adults exposed to temperatures of −10° C. at 472 mm. Hg. pressure from 15 minutes to two hours yielded variable results. It was noted that adults were generally less resistant to simulated altitude conditions than were the nymphs.

When the temperature was reduced to −15° C. with absolute pressure of 429 mm. Hg. for 30 minutes, 22 out of 24 *Oncopeltus* were found dead. At this temperature and pressure, all insects were found dead at the end of one hour.

Exposure for one hour produced paralysis in 2 and death in 20 out of 22 insects tested at −20° C. with 390 mm. Hg.; at the end of 2 hours, all were found dead. At −25° C., exposures of 30 minutes or one hour killed all insects tested.

The mortality rate was likewise 100 per cent for all milkweed bugs exposed to −30° C. and to −44° C. for 15 minutes (see Table 4).

(f) *Rhodnius prolixus* were exposed to −10° C. with absolute pressure of 472 mm. Hg. for periods of 15 and 30 minutes; these exposures resulted in death to 8 and paralysis in 8 out of 25 intects tested. At the same temperature and pressure, exposure periods of one and two hours produced death of 10 and paralysis of 2 out of 12 insects.

When the temperature was reduced to −15° C. with absolute pressure of 429 mm. Hg., exposure periods of 15 and 30 minutes

TABLE 3

The Effects of Temperature, Pressure and Time on Drosophila melanogaster

Temp. °C.	Conditions in Altitude Chamber: Absolute Pressure mm. Hg.	Corrected Rel. Hum. Per Cent	Total Exposure Hrs.	Min.	No. Flies Exposed	No. Undamaged	Results: No. Paralyzed	No. Dead
— 5	522	89	2	.	9	9	0	0
— 5	522	87	4	.	8	0	2	6
—10	472	87	.	15	18	18	0	0
—10	472	82	.	15	4	4	0	0
—10	472	87	.	30	5	5	0	0
—10	472	78	1	.	5	0	5	0
—10	472	78	2	.	19	0	19	0
—15	429	77	.	10	14	0	10	4
—15	429	83	.	15	21	5	15	1
—15	429	80	.	15	11	2	4	5
—15	429	67	.	15	32	0	18	14
—15	429	83	.	30	39	0	14	25
—15	429	71	.	30	5	0	2	3
—15	429	77	1	.	24	0	7	17
—15	429	78	2	.	20	0	0	20
—20	390	70	.	15	11	0	10	1
—20	390	74	.	30	51	0	40	11
—20	390	74	1	.	44	0	19	25
—20	390	70	2	.	15	0	1	14
—25	349	69	.	15	16	0	7	9
—25	349	69	.	30	12	0	0	12
—25	349	69	1	.	23	0	0	23
—30	310	69	.	15	20	0	0	20
—30	310	67	.	30	20	0	0	20
—30	310	70	2	.	20	0	0	20
—44	225	53	.	15	18	0	0	18
—44	225	53	.	30	12	0	0	12

produced death in 45 and paralysis in 14 out of 63 insects; only 4 were seemingly undamaged in this group. At the same simulated altitude, 15,000 feet above sea level, exposure for one and two hours produced death in 28, and paralysis in 2 out of 30 insects tested.

Further reduction of the temperature to —20° C. at 390 mm. Hg. pressure caused 20 deaths and 7 cases of paralysis of 27 *Rhodnius* exposed for 15 and 30 minutes; longer exposures invariably killed all. The nymphs had more resistance than adults to high altitude conditions.

TABLE 4

The Effects of Temperature, Pressure and Time on Oncopeltus fasciatus

Conditions in Altitude Chamber							Results	
Temp. °C.	Absolute Pressure mm. Hg.	Corrected Rel. Hum. Per Cent	Total Exposure Hrs.	Min.	No. Oncopeltus Exposed	No. Undamaged	No. Paralyzed	No. Dead
— 5	522	80	4	.	10	10	0	0
— 5	522	90	24	.	13	6	0	7
—10	472	82	.	15	17	9	3	5
—10	472	85	.	30	11	2	0	9
—10	472	78	.	30	7	0	0	7
—10	472	80	1	.	9	5	0	4
—10	472	80	2	.	11	2	0	9
—15	429	78	.	10	4	2	0	2
—15	429	84	.	15	12	2	1	9
—15	429	83	.	30	19	2	0	17
—15	429	71	.	30	5	0	0	5
—15	429	83	1	.	7	0	0	7
—20	390	70	.	15	20	2	4	14
—20	390	70	.	30	21	1	1	19
—20	390	70	1	.	22	0	2	20
—20	390	70	2	.	12	0	0	12
—25	349	74	.	15	13	3	0	10
—25	349	74	.	30	10	0	0	10
—25	349	74	1	.	10	0	0	10
—30	310	68	.	15	6	0	0	6
—30	310	68	.	30	6	0	0	6
—44	225	54	.	15	10	0	0	10
—44	225	54	.	30	9	0	0	9

Reducing the temperature to —25° C. with 349 mm. Hg. pressure was fatal to 100 per cent of all insects tested for a period of 15 minutes. Similar results were obtained in 15 minutes at —30° C. and —44° C. with pressures corresponding for standard altitudes (see Table 5).

(g) *Triatoma phyllosoma* were exposed to —10° C. with 472 mm. Hg. absolute pressure for 15 minutes, 30 minutes, and one hour; these exposures resulted in 4 deaths and 12 cases of paralysis out of 30 insects tested; 14 of this group were apparently unharmed. When the exposure period was extended to two hours at the same simulated altitude conditions, all exposed insects were found dead.

TABLE 5

The Effects of Temperature, Pressure and Time on Rhodnius prolixus

Conditions in Altitude Chamber					Results			
Temp. °C.	Absolute Pressure mm. Hg.	Corrected Rel. Hum. Per Cent	Total Exposure Hrs.	Min.	No. Rhodnius Exposed	No. Undamaged	No. Paralyzed	No. Dead
— 5	522	91	2	.	15	0	15	0
— 5	522	87	4	.	10	0	8	2
— 5	522	90	24	.	7	0	0	7
—10	472	82	.	15	13	5	6	2
—10	472	87	.	30	6	4	0	2
—10	472	78	.	30	6	0	2	4
—10	472	78	1	.	6	0	1	5
—10	472	78	2	.	6	0	1	5
—15	429	78	.	5	7	4	0	3
—15	429	78	.	10	6	3	2	1
—15	429	83	.	15	25	3	4	18
—15	429	83	.	30	26	1	4	21
—15	429	73	.	30	12	0	6	6
—15	429	80	1	.	24	0	2	22
—15	429	78	2	.	6	0	0	6
—20	390	76	.	15	14	0	7	7
—20	390	76	.	30	13	0	0	13
—20	390	65	1	.	10	0	0	10
—20	390	65	2	.	10	0	0	10
—25	349	65	.	15	16	0	0	16
—25	349	63	.	30	12	0	0	12
—25	349	64	1	.	12	0	0	12
—30	310	58	.	15	6	0	0	6
—30	310	67	.	30	6	0	0	6
—30	310	71	2	.	6	0	0	6
—44	225	54	.	15	10	0	0	10
—44	225	54	.	30	11	0	0	11

Exposure of 80 *Triatoma* to a temperature of —15° C., with 429 mm. Hg. absolute pressure for periods of 15 minutes, 30 minutes, and one hour produced death in 61 per cent and paralysis in 34 per cent; there were only 2 apparently undamaged survivors.

At —20° C. with 390 mm. Hg., 21 insects were exposed for 30 minutes; 19 were killed and 2 paralyzed. Longer exposures invariably produced 100 per cent fatality at these conditions.

Fifteen minutes of exposure killed all specimens tested at conditions simulating altitudes of 20,000 feet and higher (see Table 6).

(h) *Xenopsylla cheopis* were subjected to the following simulated altitude conditions: −10° C. with 472 mm. Hg.; −15° C., 429 mm. Hg.; −20° C., 390 mm. Hg.; and −25° C., 349 mm. Hg. absolute pressure; exposure periods varied from 15 minutes to two hours. Out of 559 fleas tested, 280 (50 per cent) were

TABLE 6

The Effects of Temperature, Pressure and Time on Triatoma phyllosoma

Conditions in Altitude Chamber								
Temp. °C.	Absolute Pressure mm. Hg.	Corrected Rel. Hum. Per Cent	Total Exposure Hrs.	Min.	No. Triatoma Exposed	Results No. Undamaged	No. Paralyzed	No. Dead
— 5	522	91	2	.	10	0	10	0
— 5	522	87	4	.	10	0	10	0
— 5	522	90	24	.	6	0	0	6
—10	472	82	.	15	12	10	1	1
—10	472	87	.	30	6	4	2	0
—10	472	78	.	30	6	0	4	2
—10	472	78	1	.	6	0	5	1
—10	472	78	2	.	6	0	0	6
—15	429	78	.	5	6	5	1	0
—15	429	78	.	10	6	0	5	1
—15	429	83	.	15	24	1	13	10
—15	429	83	.	30	26	1	11	14
—15	429	73	.	30	6	0	2	4
—15	429	83	1	.	12	0	0	12
—15	429	77	1	.	12	0	1	11
—15	429	78	2	.	6	0	0	6
—20	390	76	.	15	25	2	15	8
—20	390	76	.	30	21	0	2	19
—20	390	76	1	.	19	0	0	19
—20	390	76	2	.	10	0	0	10
—25	349	74	.	15	16	0	0	16
—25	349	63	.	30	10	0	0	10
—25	349	64	1	.	10	0	0	10
—30	310	70	.	15	6	0	0	6
—30	310	67	.	30	6	0	0	6
—30	310	71	2	.	6	0	0	6
—44	225	54	.	15	11	0	0	11
—44	225	54	.	30	10	0	0	10

found dead, 263 (47 per cent) were paralyzed, and 16 (3 per cent) were apparently unharmed.

At −30° C., with absolute pressure of 310 mm. Hg., exposures of 15 minutes and of 30 minutes resulted in death to all the fleas tested (see Table 7).

2. *The Effect of Reduced Pressure at Room Temperature on Insects. Four species of insects (O. fasciatus, T. phyllosoma, T. gerstaeckeri,* and *R. prolixus),* after 48 hours exposure in a vacuum jar at 225 mm. Hg. absolute pressure at 25° C., remained normal and active. Ten *T. gerstaeckeri* exposed at absolute pressure of 55 mm. Hg. were likewise unaffected after 24 hours at room temperature (25° C.).

3. *The Effect of Low Temperature at Standard Atmospheric Pressure on Insects.* Of 32 *T. gerstaeckeri,* exposed at −15° C. and 760 mm. Hg. absolute pressure for 15 minutes, 18 were found paralyzed and 14 were seemingly undamaged. When the time was increased to 30 minutes under the same conditions, 7 were paralyzed and 29 were found dead, of 36 insects tested. When the exposures were extended to one hour, all of the 30 *Triatoma* tested were found dead.

4. *The Effect of Relative Humidity on Arthropods.* The relative humidity in these experiments varied from 53 to 93 per cent. No conclusive evidence was obtained pertaining to the effect on arthropods of this humidity variation under the conditions of the experiments (see Tables 1 to 8).

5. *The Effects of 30,000 Feet Simulated Altitude Conditions on Experimentally Infected Insects.* Four species of insects experimentally infected with five species of microorganisms were invariably killed when subjected to a temperature of −44° C., with 225 mm. Hg. absolute pressure for various periods of time, ranging from one to 24 hours. Microscopic examination of the intestinal contents of all of these dead insects revealed viable microorganisms in some as follows (see Table 8):

(a) *Dirofilaria immitis* remained viable in *Triatoma gerstaeckeri* at the end of exposure periods of one and three hours; however, at the end of five hours of exposure, no active microfilariae were found.

(b) *Herpetomonas musca domestica* in *Musca domestica* remained alive and active at the end of one and two hours of exposure. However, at the end of four hours of exposure no viable hemoflagellates were noted in the intestinal contents of the flies.

(c) *Trypanosoma cruzi* in *T. gerstaeckeri* were found still alive at the end of periods of exposure of two and 24 hours.

(d) *Trypanosoma duttoni* in *Rhodnius prolixus* and in *T. gerstaeckeri* were found dead at the end of one and two hours of exposure.

TABLE 7

The Effects of Temperature, Pressure and Time on Xenopsylla cheopis

Conditions in Altitude Chamber						Results	
Temp. °C.	Absolute Pressure mm. Hg.	Corrected Rel. Hum. Per Cent	Total Exposure Hrs.	Min.	No. Fleas Exposed	No. Undamaged	No. Paralyzed	No. Dead
—10	472	87	.	15	17	1	0	16
—10	472	82	.	15	42	9	8	25
—10	472	80	.	30	58	3	29	26
—10	472	82	1	.	5	0	2	3
—10	472	80	2	.	30	0	9	21
—15	429	77	.	10	20	0	20	0
—15	429	83	.	15	23	0	3	20
—15	429	80	.	15	15	2	5	8
—15	429	73	.	15	20	1	16	3
—15	429	83	.	30	134	0	81	53
—15	429	83	1	.	25	0	0	25
—15	429	77	1	.	18	0	0	18
—20	390	76	.	15	34	0	20	14
—20	390	77	.	30	78	0	65	13
—25	349	69	.	15	12	0	5	7
—25	349	64	.	15	28	0	0	28
—30	310	69	.	15	17	0	0	17
—30	310	67	.	30	40	0	0	40
—30	310	70	2	.	31	0	0	31

(e) *Trypanosoma lewisi* in *T. gerstaeckeri* remained viable at the end of one and three hours of exposure. *Tr. lewisi* in *R. prolixus* remained viable for one, two, and four hour periods of exposure; however, at the end of 24 hours, negative results for active flagellates were obtained. *Tr. lewisi* in *Xenopsylla cheopis* at the end of one and two hours of exposure were all found dead.

The control experimentally infected insects for all of the above studies were kept at room temperature. Their intestinal contents were examined and invariably were found positive for active microfilariae or hemoflagellates for at least 17 hours (see Table 9).

TABLE 8

The Effects of Temperature, Pressure and Time on Microorganisms in Infected Insects

Microorganism	Exp. Infected Insect	Conditions in Altitude Chamber				Results Microorganisms	
		Temp. °C.	Absol. Press. mm. Hg.	Total Exposure Hrs.	No. Insects Exposed	Active Forms	Dead Forms
Dirofilaria immitis	Triatoma gerstaeckeri	—44	225	1	3	+	+
		—44	225	3	3	+	+
		—44	225	5	3	0	+
		+25	760	17	2	+	+
Herpetemonas musca-domestica	Musca domestica	—44	225	1	10	+	0
		—44	225	2	10	+	0
		—44	225	4	10	0	+
		+25	760	24	10	+	0
Trypanosoma cruzi	Triatoma gerstaeckeri	—44	225	2	1	+	0
		—44	225	24	2	+	+
		+25	760	24	1	+	0
Trypanosoma duttoni	Rhodnius prolixus	—44	225	1	2	0	+
		—44	225	2	2	0	+
		+25	760	48	2	+	+
Trypanosoma duttoni	Triatoma gerstaeckeri	—44	225	2	1	0	+
		—44	225	4	2	0	+
		+25	760	20	2	+	+
Trypanosoma lewisi	Rhodnius prolixus	—44	225	1	1	+	+
		—44	225	2	1	+	+
		—44	225	4	1	+	+
		—44	225	24	2	0	+
		+25	760	48	3	+	+
Trypanosoma lewisi	Triatoma gerstaeckeri	—44	225	1	2	+	+
		—44	225	3	4	+	+
		+25	760	24	2	+	+
Trypanosoma lewisi	Xenopsylla cheopis	—44	225	1	2	0	+
		—44	225	2	2	0	+
		+25	760	48	3	+	+

REMARKS: The corrected relative humidity in the altitude chamber in all of these studies ranged from 57–59 per cent in all exposures.

Discussion

Analysis of the present experimental data reveals that there are two principal factors in simulated altitude conditions which are detrimental to arthropods. One is the altitude level, with its concomitant low temperature and reduced pressure; the second is the exposure period. Thus, exposure of arthropods to simulated altitude conditions of 15,000 feet above sea level for 15 minutes produced some paralysis and death. whereas two hours of exposure at this same altitude was fatal to all species of arthropods studied.

Furthermore, conditions simulating altitudes below 10,000 feet required longer exposure periods to produce the same results. On the other hand, exposure of arthropods to simulated altitude conditions of 30,000 feet above sea level invariably killed all species of arthropods studied within 15 minutes.

Although there was some variation in resistance to a given simulated altitude among the species of arthropods, the difference was in degree rather than in kind. Thus. *Apis*, and *Musca domestica* were vulnerable at lower simulated altitudes than were the other species of arthropods. Also, nymphs of *Triatoma*, *Rhodnius*, and *Oncopeltus* were more resistant than were the adults to simulated altitude conditions. No apparent difference in altitude tolerance was noted between male and female arthropods.

The physical state of the arthropods, whether plump and recently fed or thin and hungry, had negligible effects on their resistance to simulated altitude conditions.

Variations between the altitude tolerance of arthropods and the eggs of the same species have been noted; these will be the subject of another communication.

In spite of the use of dried air in the altitude chamber, the relative humidity varied from 53 to 93 per cent. apparently without, however, modifying the results obtained.

Slight variations in the results of duplicated experiments were noted. These deviations, however, are within the limits of experimental error.

In these studies, it is apparent that the altitude factor which is chiefly responsible for the detrimental effect on insects is the low temperature (cold); apparently, reduced pressure alone is without ill effect on insects. This was illustrated in several experiments. Thus, insects exposed to −15° C. at standard atmospheric

pressure (760 mm. Hg.) were all found dead at the end of two hours, while insects exposed at 25° C. at reduced pressure (55 mm. Hg.) survived without apparent ill effect after 24 hours. These experiments were repeated several times with essentially the same findings. After further reduction to still lower pressures at 25° C., the insects were unharmed, even after 24 hours of exposure. Apparently, insects do not require the same level of oxygen partial pressure as do humans, since reduction of total pressure to this level would be fatal almost instantaneously to man. It appears that the small amount of oxygen remaining in the partial vacuum was sufficient to sustain the life of the insects. Likewise, repeated decomposition and repressurization at 25° C. had no ill effect upon insects tested.

The effect of complete removal of oxygen on insects at room temperature, at normal and at reduced pressure, is in progress; these findings will be published in the near future (Packchanian and Stevens, 1955).

Since pathogenic microorganisms remained viable in insects after exposure to conditions simulating 30,000 feet above sea level, even after 24 hours, they apparently will remain as a potential health hazard.

The experimental data shows that simulated altitude conditions of 20,000 to 30,000 feet above sea level are detrimental to insects within 30 minutes. However, it must be kept in mind that various other factors may play an important role in hastening the death or protecting the life of insects on the surface of aircraft. Since the cold in simulated altitude conditions is the major factor causing death of insects, any condition that will alter the surface temperature of the aircraft will play an important role, *e.g.*, the surface material of the aircraft, its color, the inside temperature, the location of the insect on the surface, windblast, the velocity of flight, solar radiation and heat generated by air friction, etc.

Since most commercial airplanes do not normally fly at 30,000 feet above sea level, the method described in this paper for killing the insects is obviously not suitable for general use. However, when the pilot is certain that his aircraft is infested with undesirable insects on departure, particularly during war, a flight at 30,000 feet above sea level for 15 minutes, in all probability will eliminate all viable insects on the surface of the aircraft. This altitude should be achieved as soon as possible after leaving an

arthropod infested area, well before crossing into uninfested regions. This procedure, however is an added precaution and will not eliminate the standard methods of aircraft disinsectization and disinfection on arrival at the destination or airport.

Summary

1. The following eight species of arthropods were subjected to simulated altitude conditions by exposing them in an altitude chamber equipped to produce low temperatures with corresponding reduced atmospheric pressures: *Amblyomma americanum*, *Anopheles quadrimaculatus*, *Apis mellifera*, *Drosophila melanogaster*, *Oncopeltus fasciatus*, *Rhodnius prolixus*, *Triatoma phyllosoma*, and *Xenopsylla cheopis*.

2. Most of the species tested survived two hours of exposure to conditions corresponding to an altitude of 10,000 feet above sea level. All of the arthropods studied were killed within 15 minutes at conditions simulating an altitude of 30,000 feet. Exposures at simulated intermediate altitudes produced paralysis or death in all species. The increase in death rate varied directly with exposure time, for a given altitude; or, with altitude, for a given exposure time.

3. The altitude factor responsible for the detrimental effect on insects is low temperature and not reduced pressure.

4. Microfilariae, herpetomonas, and trypanosomes in the intestines of infected insects were found to survive conditions of 30,000 feet altitude after exposure for periods ranging from 2 to 24 hours, although these conditions invariably killed insects within 15 minutes.

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CARDIOTONIC GLYCOSIDES: SOME ASPECTS OF THEIR CHEMISTRY AND PHARMACOLOGY WITH SPECIAL REFERENCE TO DIGITALIS AND ITS DERIVATIVES

H. W. Paley*

Since digitalis came into medical use as a result of the work of William Withering in 1785, a large volume of information and misinformation bearing on the subject has appeared. Only recently has much of the mystery surrounding many of the clinically observable aspects of digitalis and its derivatives been explained. What follows is an attempt to systematize certain of the facts pertaining to the cardiotonic glycosides, their chemistry, and their pharmacologic and physiologic mechanisms.

The terms digitalis (or any of its derivatives), digitalis glycoside (s), and cardiotonic glycoside (s) are interchangeable insofar as the purposes of this presentation are concerned, although many of the cardiotonic glycosides are derived from other sources than digitalis plants; *e.g.*, strophanthus, squill, Nerium oleander, convalleria, adonis, yoloxochitl, lily-of-the-valley, periploca, and ouabaio. The digitalis glycosides are extracted from the leaves of Digitalis lanata and Digitalis purpurea.

All of the cardiotonic glycosides about which the chemistry is known are classified as hydroxylactones of sterol hydrocarbons (perhydroxycyclopetanophenanthrene), *i.e.*, there is an unsaturated lactone ring replacing an hydroxyl at position C-17 of the sterol group. Replacing the hydroxyl radical at the C-3 position of the sterol group is an arrangement of monosaccharides which is characteristic for each of the groups of cardiotonic glycosides. In some of the groups of glycosides there may be other organic radicals attached to the arrangement of monosaccharides, and these too are characteristic of the particular glycoside under consideration.

Individual purified derivatives within any group of glycosides are set apart from others of that group by the arrangement of hydroxyl, alcohol or other radicals at positions other than C-3 and C-17 of the sterol nucleus. In the case of the digitalis glyco-

* From the Department of Internal Medicine, The University of Texas Medical Branch, Galveston.

sides the characteristic which separates them from all other of the cardiotonic glycosides is the nature of the monosaccharide digitoxtose. an hexose found nowhere in nature except as part of the digitalis glycosides.

As will be noted, there is a chain of three digitoxose molecules forming part of the structure of Digilanid A. All other members of the digitalis group of glycosides are also characterized by this arrangement of three digitoxose molecules, with the exception of gitalin which is characterized by only two such sugar molecules (Fig. I, a, b, c). It will be noted that attached to the last digitoxse molecule in each glycoside of the digitalis group is a molecule of glucose, except in the case of gitalin, and in the case of the purified glycosides of D. lanta there is an acetyl radical attached to the last digitoxose molecule of each of the glycosides. It is this acetyl radical which distinguishes all of the digilanids (purified derivatives of D. lanata) from the purified derivatives of D. purpurea. If the acetyl radical of each of Digilanids A and B is removed by mild alkaline hydrolysis, the resultant structures are indistinguishable from purpurea glycosides A and B, respectively (Fig. I). This does not apply to Digiland C which is peculiarly found in the leaves of D. lanata but not in those of D. purpurea. But in fair exchange D. purpurea can claim the presence of gitalin which is not found in the leaves of D. lanata. If either deacetyldigilanid A or purpurea glycoside A is now subjected to enzymatic hydrolysis and the terminal glucose molecule is removed, the drug digitoxin is formed. If this process is done with regard to deacetyldigilanid B or purpurea glycoside B the drug gitoxin is formed. In the case of deacetyldigilanid C such a process yields the drug digoxin, which because of the fact that its precursors are found only in the leaves of D. lanata, has no counterpart which can be derived from the purpurea group of glycosides. The drug gitalin is derived directly from the leaves of D. purpurea without the necessity of previous hydrolytic preparation. If any of the purified digitalis derivatives, digitoxin, gitoxin, digoxin or gitalin are now subjected to strong acid hydrolysis, the chain of digitoxose molecules are removed from the C-3 position and the aglycone or genin form of each of these respectively is formed.

It will be noted that digitoxigenin (algycone) has a methyl group at each of positions C-10 and C-13 of the sterol nucleus and in this respect is similar to the other digitalis aglycones. How-

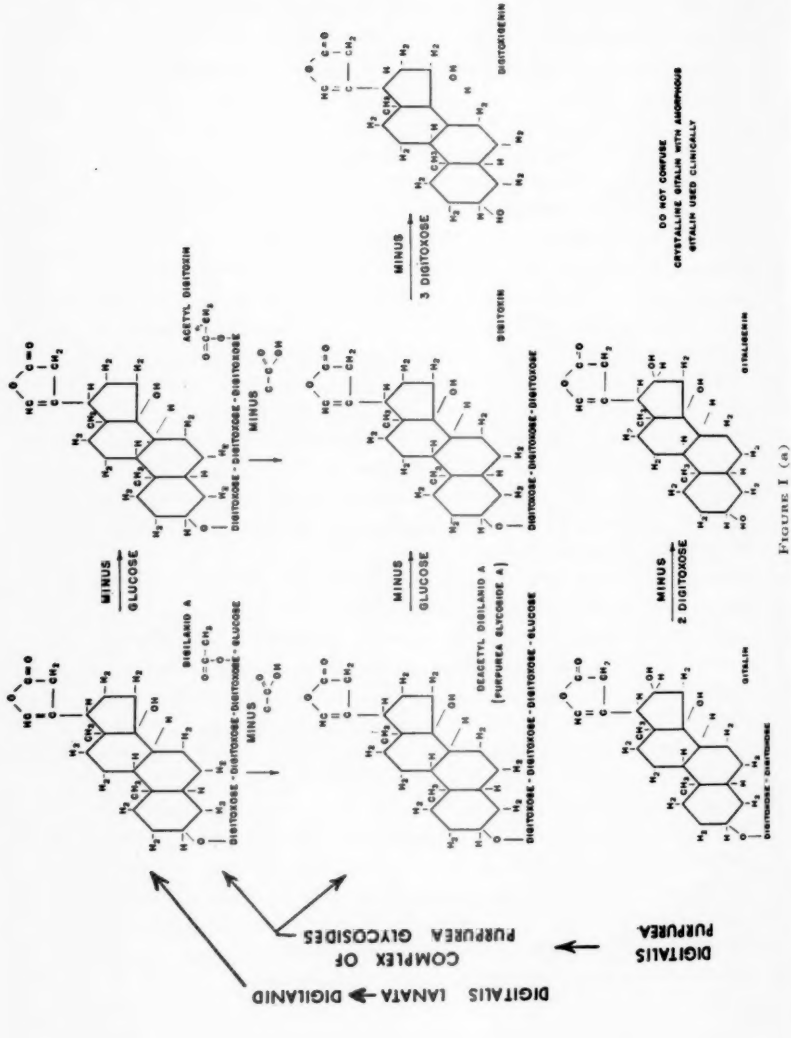
DIGITALIS LANATA → DIGILANID
COMPLEX OF
PURPUREA GLYCOSIDES
DIGITALIS
PURPUREA
DIGILANID A
DIGITOXOSE - DIGITOXOSE - DIGITOXOSE - GLUCOSE
MINUS
GLUCOSE
ACETYL DIGITOXIN
DIGITOXOSE - DIGITOXOSE - DIGITOXOSE
MINUS
DEACETYL DIGILANID A
(PURPUREA GLYCOSIDE A)
DIGITOXOSE - DIGITOXOSE - DIGITOXOSE - GLUCOSE
MINUS
GLUCOSE
DIGITOXIN
DIGITOXOSE - DIGITOXOSE - DIGITOXOSE
MINUS
3 DIGITOXOSE
DIGITOXIGENIN
GITALIN
DIGITOXOSE - DIGITOXOSE
MINUS
2 DIGITOXOSE
GITALIGENIN
DO NOT CONFUSE
CRYSTALLINE GITALIN WITH AMORPHOUS
GITALIN USED CLINICALLY

FIGURE I (a)

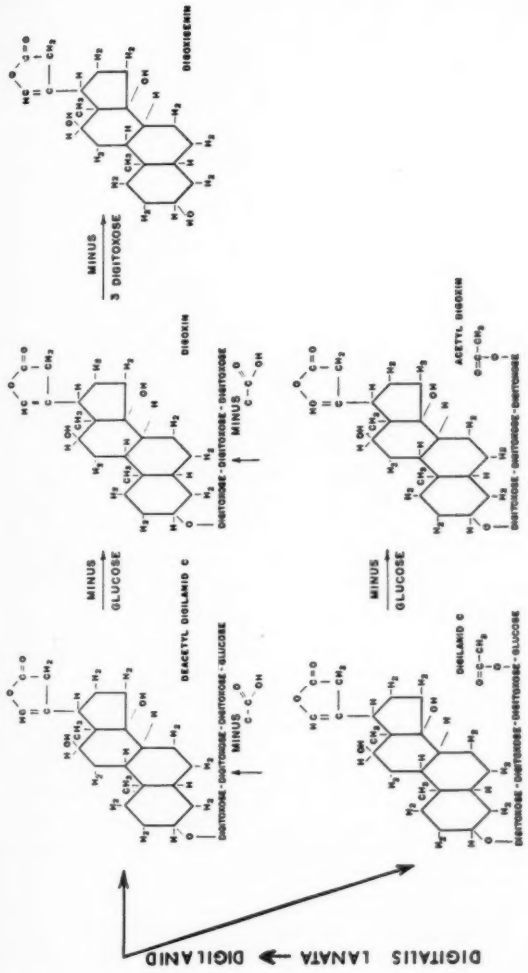
DIGITALIS LANATA ← DIGILANID
DIGITOXOSE - DIGITOXOSE - DIGITOXOSE - GLUCOSE
DEACETYL DIGILANID C
MINUS GLUCOSE
DIGITOXOSE - DIGITOXOSE - DIGITOXOSE
DIGOXIN
MINUS 3 DIGITOXOSE
DIGITOXOSE - DIGITOXOSE - GLUCOSE
DIGILANID C
MINUS GLUCOSE
DIGITOXOSE - DIGITOXOSE
ACETYL DIGOXIN
DIGOXIGENIN

FIGURE I (b)

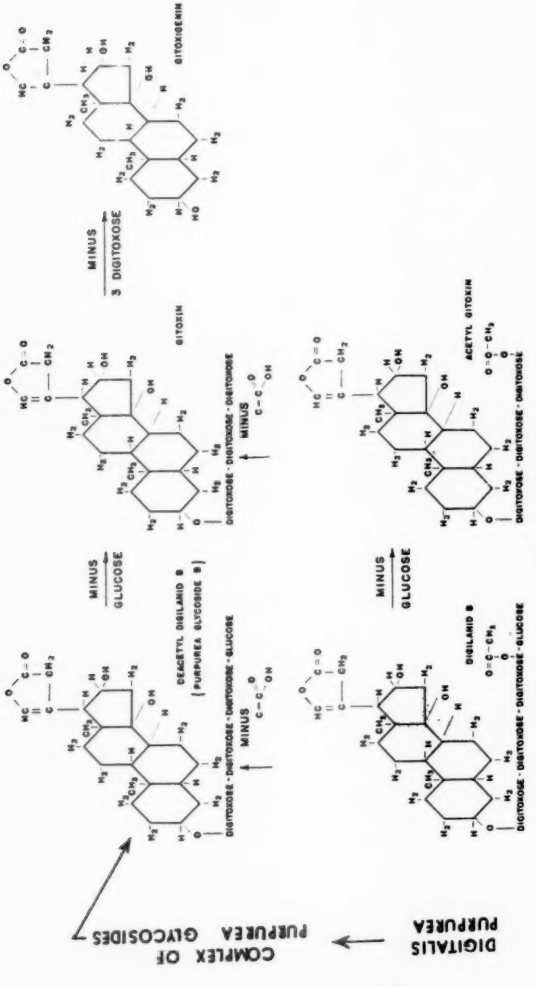
DIGITALIS LANATA → DIGILANID
DIGITALIS PURPUREA
COMPLEX OF PURPUREA GLYCOSIDES
MINUS GLUCOSE
MINUS 3 DIGITOXOSE
GITOXIGENIN
GITOXIN
DEACETYL DIGILARID B (PURPUREA GLUCOSIDE B)
DIGILARID B
ACETYL GITOXIN
DIGITOXOSE - DIGITOXOSE - DIGITOXOSE - GLUCOSE
DIGITOXOSE - DIGITOXOSE - DIGITOXOSE
DIGITOXOSE - DIGITOXOSE

FIGURE I (c)

ever, the presence of an hydroxyl radical at position C-14 in addition to the primary hydroxyl at C-3 distinguishes digitoxigenin from all other aglycones of the cardiotonic glycosides. Gitoxigenin is distinguished by hydroxyl radicals at positions C-14 and C-16 in addition to the primary hydroxyl group at position C-3. Digoxigenin is distinguished by hydroxyl groups at positions C-14 and C-12 in addition to the primary hydroxyl group at position C-3. Gitaligenin has been thought to be an hydrated form of gitoxigenin.

It is established that the portion of the general chemical structure which is cardiotonically important is the sterol hydrocarbon in association with the unsaturated lactone ring at position C-17. If the lactone ring is in any way altered the cardiotonic effect of the arrangement is supposedly lost. It has been further established that if the lactone group is given alone in the form of Angelica lactones before the administration of any of the cardiotonic glycosides, there is no activity whatsoever. When it is given along with any of the cardiotonic glycosides the specific activity of that particular glycoside is not altered. This suggests that the entire aglycone structure with the lactone ring intact is necessary for cardiotonic activity.

From the standpoint of clinical application it is interesting to note that all of the purified digitalis glycosides have activity of the same or closely similar order so that the total "digitalizing" dose of any of the glycosides is between 1.2 mg. and 2.0 mg. It is of note that the aglycone which supposedly forms the basis for cardiotonic activity is in itself considerably less active than is that particular drug from which it is derived.

As one peruses the literature regarding the many aspects of the cardiotonic glycosides it becomes apparent that aside from the chemical-structural configuration of these compounds there is little that has met with agreement. Some of the lack of understanding regarding these drugs has no doubt resulted from misleading observations made by the use of relatively crude and inaccurate methods employed in deriving information. Within the past 10 to 15 years it has become possible to measure many factors which heretofore were either calculated indirectly or were simply presumed to be true. By more recent methods certain of the former opinions have been clarified and as research progresses, less and less about this confusing subject remains a a matter of speculation.

It does not seem likely that anyone will question the fact that patients with certain forms of congestive heart failure, indeed most forms of this affliction, respond to the cardiotonic glycosides by manifesting improvement in status. Patients so treated reveal less difficulty breathing, slowing of the heart rate, diminution of edema with decrease in size of the liver (and spleen), clearing of pulmonary rales, decrease in end diastolic size of the heart, diuresis, increase in strength. Such observations following the administration of digitalis or other cardiotonic glycosides has established a general agreement that these drugs bring about an increase in the functional capacity of the heart in congestive heart failure. Although the primary organ site of action of the cardiatonic glycosides has been adequately demonstrated, the fact that a considerable controversy existed for some time is emphasized by the tendency of some to attribute the improvement brought about by the administration of these drugs in patients with congestive heart failure to changes other than effects directly upon the heart muscle.

It has been reasonably well established that the digitalis glycosides serve to enhance the contractile force of the myocardium. This observation was made at least 50 years ago by Cushney and has been confirmed by several investigators since that time. As a result of this increase in contractile force the cardiac output is increased in most cases of low output failure and in some cases of high output failure. Low output failure usually results from intrinsic cardiac lesions or from constrictions in the aorta and implies that because of diminution in the functional capacity of the myocardium it can no longer meet the minimal demands of the peripheral tissues for cardiac output. High output failure results from lesions which increase the demands of the peripheral tissues for cardiac output (thyrotoxicosis, beri-beri, arteriovenous fistula, anemia, fever, chronic cor pulmonale, osteitis deformans). It implies that the maxial effort of the myocardium can no longer meet the increased demands for cardiac output. There is also the implication that even if the cardiac output is increased by digitalis in high output failure the rate at which such an increment becomes effective is markedly diminished. It is the latter implication which may explain the failure of digitalis in many cases of high output failure. In this connection Stewart *et al.* have demonstrated the cardiac output to be increased in most cases of congestive failure but not in the normal

heart, when treated with digitalis. Harrison *et al.* have reported that by and large the cardiac output is diminished in normal hearts treated with digitalis and also in some cases of congestive failure treated similarly. McMicheal and Sharpey-Schafer noted that generally the cardiac output in congestive failure is increased by digitalis but they noted that the venous pressure first diminished in magnitude followed by an increase in cardiac output. These same authors reported that venesection alone in patients with congestive heart failure caused the cardiac output to increase and signs and symptoms of congestive failure to subside. These observations led these authors to suggest that the primary action of the cardiotonic glycosides is not upon the myocardium at all but is primarily upon a peripheral venotonic mechanism which first lowers the venous pressure in patients with congestive failure, and in so doing diminishes the right auricular pressure and the rate of inflow into the right ventricle thus reducing the rate of flow into the left ventricle, decreasing its late diastolic volume so that it becomes more effective in its force of contraction and so increases its output according to Starling's law. Dock and Tainter and Katz *et al.* agreed in principle with McMicheal and Sharpey-Schafer, but postulated a mechanism whereby there is a constriction of the hepatic veins in congestive heart failure treated with digitalis. Thus the large portal volume of blood would be dammed behind the constricted hepatic veins and the inferior vena caval volume would be relieved thus reversing the process of overdistention of the heart chambers. If the latter hypothesis is correct one would expect enlargement of the liver and spleen in patients treated with digitalis. Recently studies by Wood in which the size of the liver was measured in patients with congestive heart failure treated with digitalis, observing the immediate post-treatment period very carefully, have shown that when any change in liver size does occur such a change is a tendency toward diminution in size.

In 1950 the McMicheal group, after cardiac catheterization studies on cardiac output and right auricular pressure, conceded that their experiments in congestive failure in man using ouabain, digoxin and Cedilanid (digilanid C) demonstrated that although there is probably some slight venotonic action by the cardiotonic glycosides, there is an increase in the amount of work done by the right ventricles which is considerably greater in magnitude in those patients who were treated with cardiotonic

glycosides either with or without venesection than in those treated with venesection alone. This would leave little doubt as to the primary action of the cardiotonic glycosides.

It is an established fact that digitalization may on occasion provoke the rapid onset of pulmonary edema, or possibly the accentuation of signs and symptoms of congestive heart failure which are already manifest. To explain this several observers have noted that if digitalis is given to a patient whose left ventricle has reached its maximum capacity for output so that digitalis can no longer increase its efficiency, but that the right ventricle is yet capable of ejecting an increased volume of blood, there exists an inbalance between the output of the two ventricles. If the difference in ejection volumes is relatively great and if the pulmonary vascular control mechanism and pressure reflexes are incapable of recreating the balance, pulmonary edema and other evidences of congestive heart failure will develop. Such situations are noted most frequently in cases of severe hypertension, aortic stenosis and coarctation of the aorta. Mitral stenosis, if severe, may cause sufficient resistance to flow so that digitalis causing an increased right ventricular effort will bring about pulmonary edema.

Cattell and Gold have done experiments on the papillary muscle of the cat which indicate that the primary action of the cardiotonic glycosides is directly upon the myocardium and that the improvement in contractile force is independent of any decrease in the diastolic volume within the failing chamber (s), *i.e.*, shortening of the myocardial fibers. Pardo *et al.* have recently presented some interesting findings revealing that digitalis has a very definite effect in increasing the contractility of ischemic skeletal muscle. It was also noted by these observers that large doses of digitalis, *i.e.*, within the toxic range, caused a decrease in the contractility in response to electrical stimulation. The latter findings tend to emphasize the repeated observations of many investigators that the beneficial effects of digitalis are obtained with subtoxic or minimally toxic doses of these drugs, and that when the dose exceeds the minimally toxic range and particularly as it approaches the lethal level there is a tendency for the contractile force of the myocardium to diminish. Visscher and his group have supplied evidence to support this concept in noting that subtoxic doses of digitalis bring about an increase in both the total energy liberation of the metabolic processes within

the myocardium, and the efficiency of the myocardium to convert this metabolic energy into external mechanical work. Toxic doses, however, tend to diminish the efficiency ratio between the production and utilization of metabolic energy for purposes of improving mechanical function. Selzer *et al.* have recently reported the augmentatory effect of ouabain on the contractile force of the right ventricle of dogs in which the pulmonary artery had been markedly constricted.

The oxidation of carbohydrates within the myocardial cell provides the energy which is utilized to resynthesize phosphocreatine. Phosphocreatine in turn serves to supply the phosphate and part of the energy (if not all of the energy) to resynthesize adenosine triphosphate which is the nucleotide phosphate form which is supposed to provide phosphate and energy to the muscle fibril protein, actomyosin, which, upon becoming phosphorylated, is then in an elongated form and is endowed with the necessary energy for contraction. This entire process recurs rhythmically with each myocardial contraction. Assuming this simplified analysis to be essentially correct, it is apparent that should the chain of events be broken at any point there will be a degree of diminution of contractile power proportional to the amount of phosphate which cannot become available to the fibril protein. It has been definitely shown that the primary action of the cardiotonic glycosides is in increasing the contractile force of the myocardium. It has also been shown that if the contracting muscle is deprived of oxygen supply, either directly or by poisoning with oxidation inhibitors, the cardiotonic glycosides cannot exert their positive inotropic (energy forming) action. It has further been shown that digitalis cannot perform its beneficial effects if the heart is poisoned with dinitrophenol, for it is the action of the latter to dissociate the processes of oxidation and phosphorylation by poisoning the phosphorylation mechanism. Although when used in preparations poisoned with dinitrophenol digitalis could not perform a positive inotropic effect, it in no way effected the process of oxidation, nor did it effect the rhythmicity of impulse formation or transmission. The latter would tend to indicate that the impulse which excites contraction in normal muscle, especially myocardium, depends upon intact oxidation and not upon phosphate energy exchange.

Recently Bing and his group have demonstrated that the oxidative processes of the failing heart are normal and that the pri-

mary difficulty in the failing myocardium seems to be the inability of the fibril protein to accept phosphate and energy from adenosine triphosphate. It would therefore seem reasonable to postulate at this point that the site of action of the cardiotonic glycosides may possibly be in the improvement of the ability of the muscle fibril protein to utilize the energy rich phosphate bond.

If digitalis is given in doses which restore the balance between the oxidative processes and the utilization of the energy rich phosphate bond by improving the latter, one then observes the ideal situation in the treatment of myocardial failure. If digitalis is given in doses which exceed the subtoxic range, there is a further acceleration of dephosphorylation (utilization of the energy rich phosphate bond by Actomyosin) with compensatory increase in oxidative activity and oxygen uptake. Apparently, seriously toxic doses of digitalis are those in the range which increase the rate of dephosphorylation beyond the point at which the maximum available oxidative capacity of the heart is sufficient to produce enough energy to maintain the resynthesis of phosphocreatine. It is the latter which is sacrificed at the expense of maintaining adenosine triposphate at an effective level, *i.e.*, at a level capable of supplying the energy rich phosphate bond to the fibril protein. It is obvious that such a state of affairs will eventually lead to a tendency for adenosine triphosphate to become less readily available at which point the contractile power of the overdigitalized heart diminishes. It has been repeatedly demonstrated, however, that adenosine triphosphate is essentially normal in quantity even at the onset of digitalis induced ventricular fibrillation in animals, but the theory is confirmed by the fact that if circulation is permitted to continue by artificial means after fibrillation has begun, there will be an eventual depletion of the labile nucleotide phosphate (adenosine triphosphate) as well as phosphocratine. Thus, there being no energy rich phosphate bond available to the fibril protein, the latter can no longer relax in elongation and complete inactivity characterized by contracture is noted. The latter is never observed in patients who succumb as a result of digitalis poisoning.

Wollenburger has demonstrated that an inhibited oxidation mechanism sensitizes the myocardium to rather small doses of digitalis. This is explained on the basis that digitalis depletes the store of adenosine triphosphate. It is also true that dinitrophenol

sensitizes the myocardium to small doses of digitalis. In the latter instance adenosine triphosphate is depleted because of failure of the phosphorylation mechanism. Both of these observations lend support to the site of action of digitalis as postulated above.

The simplicity with which the above is represented is not intended to mislead one into believing that the concept of the action of digitalis or other cardiotonic glycosides is not so complicated after all. There are numerous facets of variable nature which are as yet undefined and which no doubt modify the action of digitalis to some considerable degree. Among the influences which are not well understood are those of the physiologically important cations, namely, potassium, sodium, magnesium, calcium and barium.

It is established that calcium activates the phosphorylated actomyosin fibril or at least it has some major influence which brings about the activation and contraction of this muscle protein. It is well known that calcium in great excess brings about myocardial contracture. It is noted to act in nearly every way as does digitalis. As a matter of fact, digitalis has been found to be poorly effective in the absence of at least a small amount of ionized calcium in the substrate. It is not surprising, therefore, that the conclusion has been drawn that calcium has a definite potentiating effect upon the action of digitalis. From the clinical standpoint there is a very definite synergism between calcium and digitalis. For this reason it is not wise to inject calcium rapidly into the circulation, even of a normal individual, and it may be quite dangerous to give calcium at all to some patients who have been digitalized. Death has been observed to quickly follow the administration of calcium to digitalized patients. It has been noted that dogs made hypercalcemic by treatment with parathormone are unusually sensitive to the cardiotonic glycosides. Since acidosis, primary or secondary hyperparathyroidism and parenteral administration of calcium increase the ionized calcium level in the plasma, it is not unreasonable to assume that such circumstances have some effect in increasing the sensitivity of the myocardium to digitalis.

Barium is not naturally available within the electrolyte pattern of man but it is more toxic than is calcium and acts in an identical manner when introduced into the electrolyte pool.

That there is a loss of intracellular potassium in heart failure has been established and this is certainly not surprising when one

considers the fact that potassium is the principal cation involved in the oxidation—phosphorylation exchange mechanism. It simply follows that if there is a loss of myocardial phosphate and creatine in congestive heart failure one might also expect potassium to be lost. The fact that potassium is not selectively retained by the renal tubules as is sodium explains the increased amount of potassium found in the urine of patients with congestive heart failure. The cellular membrane is ordinarily considered to be only slightly permeable to sodium from without inward and to potassium from within outward, however this is no doubt due to the cycle of potassium utilization within the cell leaving the latter cation unavailable to permeate the membrane in any considerable amount. The Donnan theory of membrane equilibrium would then explain why sodium is not needed within the cell and so does not migrate in that direction. In congestive heart failure, however, the loss of potassium from within the cell upsets membrane ionic equilibrium and sodium migrates to replace the lost potassium within the cell. Water also enters the cell to maintain osmotic balance with the extracellular space which because of renal retention of sodium is overloaded with that cation plus water. In terms of overall electrolyte and water metabolism within the body this leads to increased body weight (water retention) followed by an increase in plasma volume. This in turn causes an increase in venous pressure and an increase in the diastolic filling of the heart chambers. The myocardium thus overdistended cannot work efficiently and the cardiac output is further decreased. This is obviously a vicious cycle.

Recent work with radiochromium tagged erythrocytes in the study of blood volume indicates that the blood volume may not be appreciably increased in congestive heart failure. However, Eisenberg has shown that a definite increase in blood volume occurred in his series. He was able to correlate these findings with heart size but not with venous pressure.

In a patient with congestive failure the cycle of circulatory deterioration can be interrupted by the use of digitalis, which by acting at the site postulated earlier, reverses the process so that there is an increase in intracellular potassium secondary to improvement in the metabolism of the energy rich phosphate within the myocardium. Again in terms of overall electrolyte and water metabolism, there is then a migration of sodium and water outward and since osmotic balance must again be restored

there is a diuresis of sodium and water. The diuresis which occurs has been attributed to the influence of renal tubular function rather than to an improvement of glomerular filtration efficiency, although Merrill and others believe the latter is largely responsible. It has been noted in patients with congestive heart failure who have normal kidneys that glomerular filtration rate is little changed if any during the period of decompensation. This does not imply, however, that the cardiotonic glycosides improve directly anything other than myocardial function.

If digitalis is administered in quantities beyond the mildly toxic therapeutic dose it would follow that there would again be a loss of potassium from within the cell. It is during this later phase of digitalis intoxication that the characteristic electrocardiographic changes become pronounced.

It has been repeatedly observed that ventricular arrythmias ranging from extrasystoles to ventricular tachycardia which occur during the toxic phase of digitalization are markedly diminished or completely abolished at least temporarily by the administration of relatively large doses of potassium. Magnesium has an action very similar or identical to that of potassium and has been used intravenously and directly into the heart to restore normal rhythm in patients with arrythmias due to digitalis toxicity.

It now becomes apparent that the action of the cardiotonic glycosides is in some respects quite similar to that of desoxycorticosterone. Like desoxycorticosterone, subtoxic levels of digitalis protect the intracellular content of potassium, although there is considerable evidence that desoxycorticosterone acetate reduces intracellular potassium. It has been observed that digitalis is capable of keeping adrenalectomized animals alive for relatively long periods of time in a manner very suggestive of the action of desoxycorticosterone. These similarities are not surprising when one notes the similarity in the chemical structures of the two.

There are also suggestions of similarities to other hormones. Le Winn has reported several cases of gynecomastia in males resulting from digitalis in patients who had sustained previous liver disease subsequent to congestive heart failure.

What happens to digitalis or to any of the cardiotonic glycosides after they are introduced into the metabolic pool of the body? Until recently it bas been extremely difficult to accurately ascertain the fate of the cardiotonic glycosides in the body by

bioassay methods. Use of the embryonic duck heart has made such determinations considerably more reliable, however, with the advent of radioactive digitoxin even the most recent bioassay methods are relatively crude. Radioactive digitoxin has been extracted from the leaves of D. purpurea which has been grown in an atmosphere of pure $C^{14}O_2$ by the Geiling group at the University of Chicago. Practically all of the published work with this substance has been done by this group of investigators.

If a digitalizing dose of digitoxin is administered intravenously into a digitalis sensitive animal, there is no evidence of the drug in the blood within one hour or even less following the injection. The substance rapidly diffuses into the extra-cellular space and then becomes "fixed" to the proteins of the various tissues. It has been noted that the hearts of digitalis sensitive animals "fix" radioactive digitoxin to a much greater degree than does the cardiac tissue of non-sensitive animals such as the rat or the rabbit. It has also been shown that in animals which tend to excrete digitoxin in large amounts by way of the urine, there is a large amount of radioactive digitoxin "fixed" to the kidney following administration of the drug. Evidence is available that in man no more than 10 per cent of digitalis is destroyed or excreted by the liver. Other studies with radioactive digitoxin in man reveal that 60 per cent to 80 per cent of the amount of digitalis excreted by way of the urine. These observations are in some considerable conflict with former concepts that the major route of excretion of digitalis in man is by way of the liver and gastrointestinal tract. In other animals, the rat and the rabbit for instance, the major route of excretion of radioactive digitoxin is by way of the liver and the gastrointestinal tract.

It is interesting to note that only about 10 per cent of the radioactive material excreted by man is in the form of unchanged digitoxin. The remainder is excreted as metabolic products of digitoxin, including radioactive urea. The latter finding lends support to those who contend that digitalis in the process of being metabolized becomes part of protein molecules, possibly an enzyme system of some kind.

The concept of digitalis accumulation within the body is supported by the fact that it has been noted that unchanged radioactive digitoxin is excreted for as long as 6 weeks following a digitalizing dose of the drug. Metabolic products have been noted in the urine for as long as 12 weeks following such a dose. In this

connection, however, it is to be noted that there is a rapid excretion of the drug during the first 3 days following its administration with about 20 per cent of the total excretion occurring within that time, and about half of this 20 per cent within the first 24 hours. The rate of excretion then appears to level off 6 to 8 days following administration. It is also interesting to note that there is no uniform rate of excretion of digitalis as was formerly supposed, but that the rate of excretion depends upon the dose of the drug and the level attained in the fluid and tissues of the body.

It has recently been shown that individuals with left ventricular failure are able to excrete digitalis by the urinary route just as efficiently as are normal persons given a similar dose of the drug. If, however, there is right venticular failure and edema there is a delay of about 24 hours before the rate of excretion equals that of the normal. Whether this delay is dependent upon improvement in renal function subsequent to a relief from congestion, or whether it is dependent upon the mobilization of extracellular fluid in which the digitalis has become diluted, is not known. For many years it has been assumed that the latter mechanism is responsible. However, Friedman *et al.* have demonstrated by use of the embryonic duck heart assay method that only a minute quantity at most is found in edema fluid or other fluid collections in digitalized patients. In this connection it is of note that not infrequently edematous individuals who have been digitalized, particularly those who have become edematous while taking digitalis, and who then manifest diuresis, develop signs and symptoms indistinguishable from mild to moderately severe digitalis intoxication. Levine *et al.* have noted that influences of electrolyte balance upon the effect of digitalis may cause apparent toxic effects from a relatively small dose of the drug. For this reason it may be wise to first treat patients with congestive heart failure with bedrest, salt poor diet, free fluid intake and diuretics. The use of digitalis will then not entail some of the risk otherwise observed.

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THE EVOLUTIONARY DYNAMICS AND TRENDS OF EXFOLIATIVE CYTOLOGY

GEORGE N. PAPANICOLAOU*

The study of cells exfoliating from the surface of the epithelial lining of various organs of the body and the investigation of their significance in the diagnosis of malignant and other pathologic conditions has received great impetus in the past few years.

"Exfoliative Cytology," as this rapidly developing offshoot of General Cytology is known today, is now recognized as a sound and reliable tool for diagnosis and research and is widely used in the United States and other countries throughout the world.

Its successful application in the discovery of incipient or hidden carcinomas of the uterine cervix and of other organs has proved it to be particularly suitable for mass screening in cancer detection clinics.

The recognition of Exfoliative Cytology as an adjunct to histopathology is evident from the increasingly important place assigned to it in pathologic and other diagnostic laboratories and by its inclusion as a requirement of the American Board of Pathology.

The recent establishment of two organizations, the Inter-Society Cytology Council and the Southern Society of Cancer Cytology, is proof of its mounting appeal to a growing circle of medical and biological scientists. Its significance in the control of cancer is evidenced by its endorsement and support by the National Cancer Institute, the American Cancer Society, the Commonwealth Fund, and many local and state Public Health Services and private welfare organizations.

Courses in Exfoliative Cytology are now offered in several institutions, and the number of original articles, monographs and books published on this subject during the last decade has long passed the 1,000 mark.

These achievements appear quite remarkable when one considers that they have been made within the short period of 10 to 12 years. The rapid growth and expansion of Exfoliative Cy-

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tology during this time is in sharp contrast with its relatively slow development over the much longer period of the previous ninety some years.

It was back in 1847 that Pouchet published his classical studies on ovulation and other related phenomena. Although handicapped by the lack of efficient optical equipment and adequate technical procedures, he gave an elaborate description of the cellular make-up of the vaginal fluid and its cyclic modifications during the various stages of the menstrual cycle in women.

Since then many investigators have made a special study of the exfoliated epithelial cells which are always present not only in the vaginal secretion, but also in urine, sputum and other fluids of the body. A large number of significant and lasting contributions were made during these many years, especially by pathologists who turned their attention to the problem of diagnosing cancer through the identification of desquamated epithelial elements. The accumulated mass of evidence in favor of this diagnostic approach did much to affirm its basic soundness and value, yet failed to inspire full confidence in its practicability and dependability as a routine diagnosis procedure.

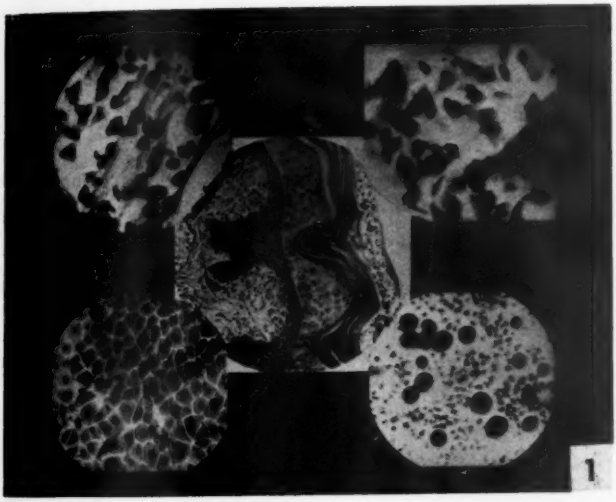
It was at this stage of an apparent stalemate that a forceful movement made its appearance like a new tributary flowing into an old stream and helping it to gather new strength to surmount the obstacles which had blocked its further advancement.

This movement had its source in a basic morphologic study which originally was unrelated to the problem of cancer and its diagnosis by cytologic criteria. In the course of an investigation carried out about 38 years ago by the late Professor Charles R. Stockard and myself, the vaginal smear was used as a method of determining the sequence of cyclic morphologic changes in the reproductive organs of the female guinea pig (Stockard and Papanicolaou, 1917).

With the aid of this simple smear technique and improved methods of fixation and staining, it became possible to establish the existence of distinct cytologic patterns appearing in an orderly and rhythmic fashion in the vaginal smears of this animal. Since these patterns reflected periodic morphologic and physiologic changes occurring not only in the vagina, but also in the uterus and ovaries, the smear technique was found to be very appropriate for the study of problems pertaining to the mor-

phology and physiology of the sex organs and their cyclic modifications.

Figure 1 shows four distinct cytologic patterns appearing in sequence in the vaginal smears of a guinea pig. Each pattern typifies a different stage of the estrous cycle and, therefore, a different functional state in the organs of the female genital tract.



The change from the upper left to the upper right indicates the appearance of the heat period or estrus, *i.e.*, the peak of ovarian follicular activity. The cells of these two patterns are exfoliated from the more superficial epithelial layers, as shown in the cross section of the vagina in the center. The cells of the two lower patterns are derived from the deeper layers of the vaginal epithelium and mark the completion of the ovulatory process and the ensuing termination of the estrous stage.

The vaginal smear, as perfected in the course of these studies, soon found general application. The sex cycle of other rodents, as the mouse and rat, and of several higher mammals, such as opossum, sow, Macacus rhesus as well as of other monkeys were studied and more minutely analyzed with the aid of this technique (Allen, 1922; 1927; Corner, 1921; Hartman, 1923; 1932; Long and Evans, 1922).

The establishment of a regular sex cycle in the guinea pig, mouse and rat proved to be of immense value in investigations and experimental studies on the endocrine glands and their specific action upon the female reproductive organs. It also opened the way for new and significant observations and discoveries in the biochemistry of sex hormones. The isolation of the estrogenic hormone was accomplished through the evaluation of its effects on spayed mice with the aid of vaginal smears (Allen and Doisy, 1923). The knowledge and understanding of the hormonal influences exerted by the pituitary gland were greatly widened. The studies on Macacus rhesus and other primates deepened considerably our insight into the factors regulating follicular growth and ovulation in the human ovary.

The application of the vaginal smear to the study and analysis of the sex cycle in women has been another major contribution. It has led to many significant observations related to the morphologic and physiologic changes occurring periodically in the ovary, uterus, and vagina of women and to the regulatory action of the sex horomones.

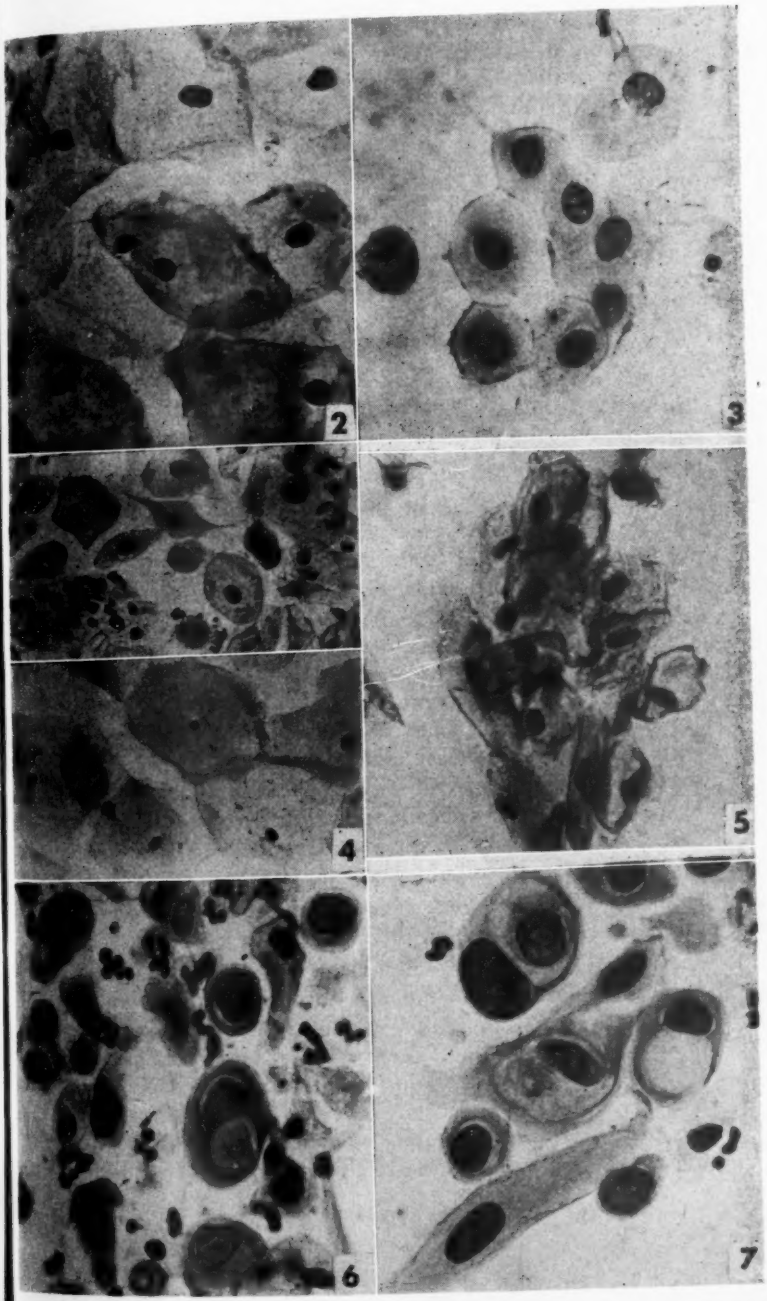
The examination of vaginal smears has thus become a very advantageous tool for the evaluation of the normal human menstrual cycle (Papanicolaou, 1933) and of such functional states as amenorrhea and menopause.

The cells illustrated in figure 2 are superficial squamous cells from a vaginal smear of a normal woman. The smear was taken on the 13th day of the menstrual cycle. The type of the cells and their pronounced cornification indicate a high ovarian follicular activity.

The cells in figure 3 are from a vaginal smear of a 59-year-old post-menopausal woman. They are characteristic of a very low ovarian functional state, as found after menopause or in primary amenorrhea.

The smear technique proved to be valuable also in the study of problems related to sterility and in the appraisal of the therapeutic action of various hormones, particularly the estrogens. The marked change in the cytologic pattern of the vaginal smear caused by the administration of estrogenic preparations permits, in fact, an objective and accurate evaluation of their physiologic action (Papanicolaou and Shorr, 1936).

This is illustrated in figure 4. The upper half shows a post-menopausal smear from a woman 58 years old. The lower one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illustrates a smear taken from the same woman following treatment with an estrogen. (In this slide the magnification is somewhat lower than in all other figures, in which a uniform magnification was maintained for the sake of comparison.)

Another field opened to exploration by this method was pregnancy. The appearance of fairly characteristic patterns in the vaginal smears of women during normal gestation and in threatened, missed or incomplete abortions and ectopic pregnancies makes possible a cytologic appraisal of these conditions (Papanicoloau, 1925).

The cells illustrated in figure 5, especially those with an oyster shell form, are characteristic of a normal gestation.

Even the vaginal smear of a newborn baby has its own distinctive morphologic traits reflecting the influence of the maternal hormones, particularly that of the estrogenic hormone (Papanicolaou and Fraenkel, 1938).

The most significant and fruitful utilization of the cytologic method has undoubtedly been the one related to the study of the exfoliative cytology of cancer. When the vaginal smear technique was first applied on a large scale in the study of the menstrual cycle of women at the Woman's Hospital of New York City in 1923, it was felt that the exploration should not be limited to the examination of smears from normal women alone, but that it should be extended to patients with different pathologic conditions, including cancer. The first observation of cancer cells in a smear of the uterine cervix was one of the most thrilling experiences of my scientific career. The striking morphologic characteristics of cancer cells and of their nuclei could not possibly fail to impress anyone seeing them for the first time.

Figures 6 and 7 show a variety of aberrant cell forms from a case of a squamous cell carcinoma of the cervix. The nuclear abnormalities are particularly prominent.

As more cases were examined and the cytologic observations found a confirmation in the operative and pathologic findings, the practical usefulness and reliability of this diagnostic approach became apparent. These early studies were reported in 1928 (Papanicolaou, 1928) in a brief preliminary paper, which, however, failed to convince my colleagues of the practicability of this procedure. The prevailing opinion, as expressed by one of the outstanding pathologists of that time was that since the uter-

ine cervix was accessible to diagnostic exploration by biopsy, which is a relatively simple procedure, the use of the cytologic examination of vaginal smears appeared to be superfluous.

This opinion reflected the view of the large majority of pathologists and other medical men of that period. To obtain smears for examination was a favor granted mainly for the purpose of research. Cytologic reports that might conflict with clinical or pathologic findings were virtually ignored. This discouraging situation caused a shift in the direction of my research for the next ten years toward other possible applications of the cytologic method such as the aforementioned studies of the action of the sex hormones upon the accessory organs of reproduction and its evaluation by cytologic criteria. This work was conducted in cooperation with Dr. Ephraim Shorr of the Department of Medicine of Cornell University Medical College. It resulted in the recognition of certain cytologic patterns in the vaginal smears which proved to be very useful for evaluating the effect and the therapeutic action of some of the sex hormones (Papanicolaou and Shorr, 1936; Papanicolaou, Ripley and Shorr, 1939).

The turning point in the attitude of the medical profession toward Exfoliative Cytology came about in 1943. It was then that the results of a more exhaustive study of the value of the vaginal smear in the diagnosis of cancer of the uterus, carried out over a period of 3 years at the Department of Gynecology of Cornell University Medical College and the Lying-In Hospital, were published in a monograph by Herbert F. Traut and myself (Papanicolaou and Traut, 1943). These observations were soon confirmed and the method was endorsed by Joe V. Meigs. Ruth M. Graham, Maurice Fremont-Smith and associates of the Harvard Medical School and the Vincent Memorial Hospital in Boston (1943) and by Theodore Neustaedter and Locke L. Mackenzie of the New York Post-Graduate Medical School (1944). Once the ball started rolling it constantly kept gathering speed and more strength imparted to it by the many contributions of gifted investigators who devoted their talents to this field.

The revived interest in and ultimate acceptance of this diagnostic approach can be related to two decisive factors. One was the introduction of improved and more adequate technical procedures. The immediate fixation of the smears was an important step, as it provided a better preservation of the exfoliated cells. Air-dried cells become distorted to a point of a virtual effacement

of their fine structural details. It would not be an exaggeration to state that had dry instead of fixed wet smears been used in the original studies Exfoliative Cytology as we know it today would probably be nonexistent. The introduction of alcoholic stains which provide more translucent coloration of the cells and of staining procedures more specifically adapted to the staining of smears has also greatly added to the diagnostic efficiency of the cytologic test (Papanicolaou, 1942).

The second significant factor was that a basic knowledge of the normal exfoliative cytology of the female genital tract had been acquired during the above-mentioned studies on the human sex cycle. The abnormal features of exfoliated malignant cell forms could not have been evaluated adequately without the yardstick provided by a thorough knowledge of the corresponding normal cell types. Normal cytology is to malignant cytology what histology is to histo-pathology.

Perhaps the most productive application of the smear technique has been in the diagnosis of cancer of the cervix. There is hardly another organ from which cytologic and histopathologic material can be obtained with greater ease. The study of such material and the correlation of cytologic with histopathologic findings have been largely responsible for the unusually rapid advancement of our knowledge in the malignant cytology and histopathology of this organ. The progress was most notable in the study of early malignant lesions brought to light by the examination of vaginal and cervical smears.

The basis for this lies chiefly in the fact that there is a continuous shedding of cells from the surface of all epithelial linings which is apt to be more copious in cancer as a result of the high proliferative activity of the malignant cells. Exfoliated cancer cells may thus be observed in smears even in the earliest developmental stages of a malignant neoplasm. It is because of this feature that the detection of a cancerous growth in its incipiency and prior to invasion and to the appearance of overt clinical symptoms, can be achieved in the uterine cervix and many other organs more efficiently by this than by any other method of diagnosis.

The great number of intra-epithelial carcinomas or carcinomas-in-situ thus far uncovered have provided the pathologist with excellent material for the study of the histogenesis of cer-

vical carcinoma, and the clinician with the opportunity to attack cancer more efficiently at an early, curable stage.

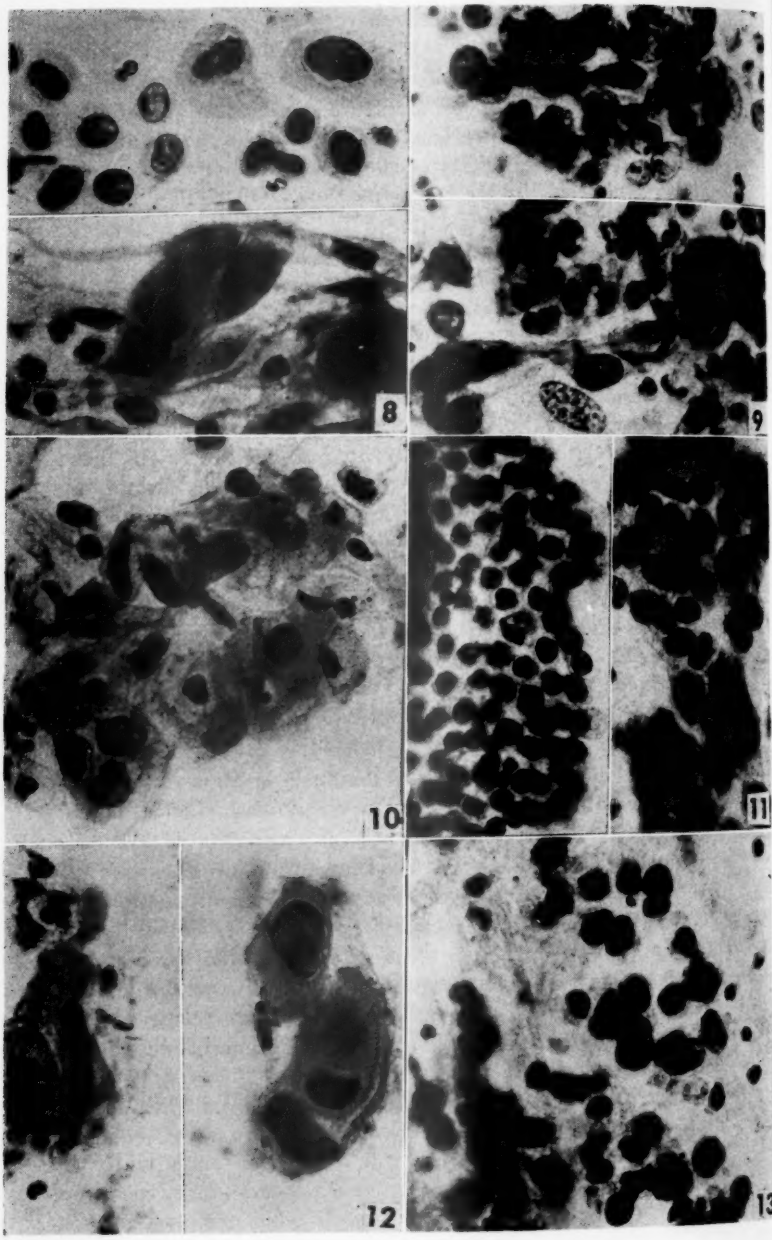
Another significant contribution of the cytologic method has been the recognition of a number of distinct smear patterns which add greatly to the diagnostic as well as the prognostic value of the method. The type, stage and developmental potentialities of a cervical carcinoma can thus be more intelligently evaluated. Certain patterns appear to predominate in the advanced stages indicating the presence of an invasive carcinoma. Others are encountered in the early developmental stages, more frequently in carcinomas still confined within the epithelium.

The upper half of figure 8 shows a carcinoma-in-situ, and the lower an invasive carcinoma of the cervix. Their cytology is of the squamous type, and indicates an ectocervical origin. Both exhibit malignant characteristics, chiefly abnormal nuclear features. However, the cells of the carcinoma-in-situ retain their individuality and to a large extent their similarity to normal cervical cells of the parabasal type; whereas those of the advanced invasive carcinoma are more densely grouped and show more abnormal and bizarre forms differing from all normal types.

The two smear patterns illustrated in figure 9 are also from a carcinoma-in-situ (upper) and an advanced invasive carcinoma (lower) of the cervix, but their cytology differs from that shown in figure 8 as it suggests an endocervical rather than an ectocervical origin.

Marked nuclear abnormalities may also be observed in cells derived from the more superficial layers of the squamous epithelium of the ectocervix.

The abnormal cytologic pattern shown in figure 10 represents a rather superficial lesion often found in cases with an ambiguous pathologic picture. Pathologists do not always agree as to its malignant nature. Though some cases exhibiting this pattern have been diagnosed pathologically as carcinoma-in-situ, others have been interpreted as dysplasia, metaplasia or basal hyperactivity. A subsequent disappearance of all abnormal cells from the smears has also been observed in cases of this type, particularly during pregnancy, when the occurrence and spontaneous regression of this aberrant superficial cell pattern have been most frequently noted.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All these observations raise many questions which cannot be answered at present, yet act as a challenge and open new avenues of thought to the many investigators who are now engaged in the study of problems related to early malignancy and especially to carcinoma-in-situ.

A detailed account of these special studies may be found in the many articles, monographs and books dealing with this subject. Only a brief discussion will be given in this presentation.

Endometrial cytology has been much more thoroughly explored since the endometrial aspiration technique has come into wider use. Smears prepared from fluid aspirated directly from the intra-uterine cavity, provide particularly suitable material for the diagnosis of endometrial adenocarcinomas, thus improving considerably the accuracy of the cytologic test and the prospects of a much earlier detection of endometrial cancer.

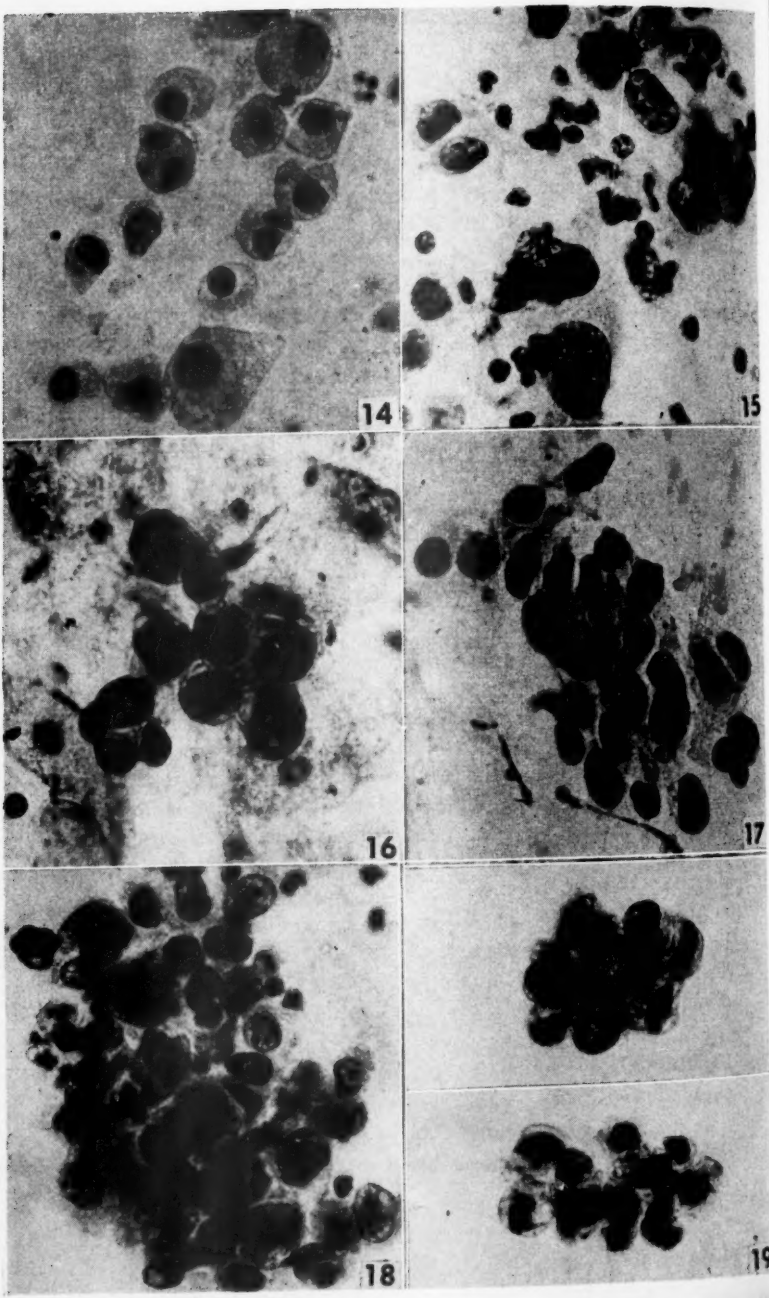
Figure 11 shows side by side one cluster of normal and one of malignant endometrial cells from an endometrial smear of a case of adenocarcinoma of the endometrium. The abnormal features of the malignant cells are apparent.

In the respiratory system the diagnostic value of the cytologic method received early recognition. The examination of sputum and of bronchial aspiration or washing specimens is now a generally accepted laboratory procedure. Its diagnostic value has been reported as even greater than that of bronchoscopic biopsy. Many unsuspected or hidden carcinomas of the lung have been detected by routine cytologic examination. The exfoliation of malignant cells within the bronchi is usually copious in the primary carcinomas of the lung and their identification is no more difficult than in smears from other organs. In some cases, it is, in fact, possible to recognize not only the presence of a malignant neoplasm but also its type.

In the bronchogenic epidermoid carcinomas (Fig. 12) exfoliated malignant cells capture the eye of the microscopist by their distinctly abnormal features and their strongly acidophilic or orangeophilic reaction.

In anaplastic carcinomas such as the oat cell carcinoma (Fig. 13) the cells are much smaller and their nuclei highly hyperchromatic.

In the adenocarcinomas (Fig. 14) the glandular origin of the exfoliated malignant cells is often indicated by the vacuolation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of the cytoplasm, the eccentric position of the nucleus and the grouping pattern of the cells.

In the gastro-intestinal tract very good results have been obtained in the diagnosis of carcinomas of the stomach particularly with the use of the gastric balloon or other techniques by which adequate and well preserved material for a cytologic study can be procured.

Figure 15 shows cells of a carcinoma of the stomach from a gastric specimen obtained with the balloon technique.

The cytologic examination of esophageal and rectal or colonic washings for the diagnosis of malignant neoplasms of the esophagus or lower intestine have also proved very rewarding.

Figure 16 illustrates a cluster of malignant cells from a carcinoma of the esophagus found in a smear prepared from a esophageal washing specimen.

Cells of an adenocarcinoma of the rectum from a specimen of rectal washing are shown in figure 17.

In the urinary and male genital tract the method has given especially good results in papillary and other tumors developing in the transitional epithelium of the urinary bladder or the pelvis of the kidney.

Figure 18 illustrates a cluster of cells of a carcinoma of the bladder from a centrifugated specimen of bladder urine.

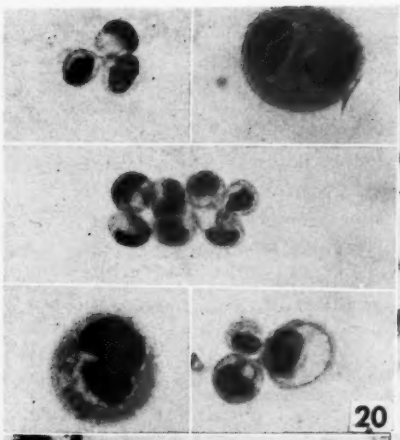
Some of the earliest known cases of carcinoma of these organs have been detected by microscopic examination of urine catheterized from the bladder or the ureter.

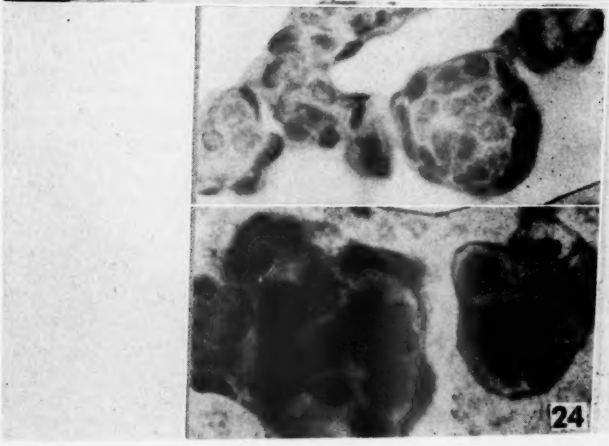
The two clusters of malignant cells shown in figure 19 are from a case of a very early carcinoma of the pelvis of the kidney detected at an operation performed on the basis of repeated positive ureteral and bladder urine specimens.

In carcinoma of the kidney and the prostate we are still handicapped by the lack of effective technical procedures for obtaining adequate material for cytologic study. However, the method can be used to advantage in the diagnosis of malignant neoplasms of these organs.

Figure 20 shows cells of an intramural carcinoma of the kidney from a ureteral urine specimen.

Figure 21 illustrates a large cluster of cells of an adenocarcinoma of the prostate found in a voided urine specimen taken after prostatic massage.





In pleural, peritoneal and pericardial exudates, and in fluids aspirated from other body cavities or cysts, the exfoliating cells may provide evidence of a primary or metastatic malignant lesion. The particularly favorable nutritive environment provided by the exudates, explains the extremely active growth and proliferation often exhibited by the malignant cells within the pleural peritoneal and pericardial cavities.

In the case of metastatic cystadenocarcinoma of the ovary illustrated in figure 22 the smears prepared from the peritoneal fluid were virtually loaded with malignant cells and cell clusters.

A more recent application, which appears to be very promising, is that of the cytologic evaluation of breast secretion.[1] The results are especially gratifying in the diagnosis of papillomas or papillary carcinomas developing in the larger ducts of the mammary gland.

The large cluster of cells on the upper side of figure 24 is from a breast secretion smear of a woman with a benign papilloma of the breast. The two lower clusters of distinctly malignant cells are from a breast secretion smear of a case of papillary carcinoma.

The early appearance of a relatively profuse exfoliation in such cases enhances the diagnostic value of the method and the detection of incipient malignant lesions prior to the appearance of a palpable mass or to a lymph node involvement. Several unsuspected carcinomas-in-situ or very early ductal carcinomas of the breast uncovered by the routine examination of breast secretion are already on record.

The malignant cells shown in figure 23 are from a breast secretion smear of a very early intraductal carcinoma diagnosed pathologically as carcinoma-in-situ. No palpable mass was noticeable in the breast and there was no evidence of metastasis to lymph nodes. The exploratory operation, which disclosed the presence of this early malignant lesion, was decided upon on the strength of the conclusively positive cytologic findings.

The application of this diagnostic procedure in the breast is at present limited by the difficulty in obtaining adequate secretion for cytologic study. Should this handicap be overcome, a much

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[1] The author gratefully acknowledges the aid granted by the American Cancer Society for the investigation of the expoliative cytology of the breast, uterus, and other organs.

wider use of this method in the diagnosis and control of cancer of the breast by mass screening may be anticipated.

Research in several fields of Biology, Biochemistry, and Medicine has been greatly stimulated by the utilization of the cytologic test. The knowledge of the action and effect on cancer and other diseases of many drugs and of various modes of therapy such as irradiation has been greatly advanced by the use of this relatively simple procedure which permits day by day observations. The method lends itself superbly to the following of patients subjected to surgery or medical treatments for checking the extent and duration of the improvement and the eventual recurrence of the disease.

Recent work by Ruth Graham and her associates (1953) indicates that the vaginal smear may possibly be used prognostically to determine in advance whether a given cancer of the cervix will respond better to irradiation or surgery.

Future investigation by the cytologic method will probably be directed more energetically toward the study of the morphologic changes and aberrations which precede and possibly pave the way to the appearance of cancerous lesions. The metaplastic changes occurring in the lung and other organs may be cited as an example.

The enumeration of these many ramifications of exfoliative cytology may serve as a measure for appraising its evolutionary dynamics and its developmental potentialities and trends.

If in this, my response to your kind invitation, I have given major emphasis to the more practical aspects of exfoliative cytology, I have, I believe, not entirely overlooked the value of a "pure" science that implies the freedom of the investigator to follow a hunch without considering whither his studies may lead.

Indeed, I feel that the story just unfolded, while necessarily somewhat personal, does illustrate the value of basic research, which, in this practical age is not always given its rightful share of recognition. It also shows how a study of purely academic interest has blossomed into a variety of useful applications from more efficient animal breeding to preventive medicine.

Thus the evolution of Exfoliative Cytology may well deserve to rank with certain other scientific developments of the past as an example of "serendipity"—that picturesque word-invention of Horace Walpole, who thereby sought to designate a phenome-

non of scientific advance, which others have called "the happy accident."

The purpose of the initial study which led to the use of the vaginal smear technique in the analysis of the female sex cycle first in rodents and then in higher mammals and man was indeed entirely unrelated to the detection of cancer or to any of the subsequent practical applications of this technique. The original investigation was planned as a basic and purely morphologic study of the chromosomal complex, more specifically of the sex chromosomes in the female guinea pig.

The ovarian material needed for this study could be obtained only during a certain stage of the estrous cycle of this animal, specifically about the time of ovulation, when the maturing ova go through the meiotic processes leading to the final reduction in the number of the chromosomes.

An urgent need was thus created for a method by which the various stages of the estrous cycle and of the exact time of ovulation could be accurately determined. This resulted in a persistent mental quest which was finally crystallized in a rather simple thought. "Since the females of all higher mammals are known to have a periodic menstrual discharge, lower mammals, such as the rodents, should also have one, but probably too scanty to be evidenced externally."

It was this thought which prompted the daily examination of the vagina of a number of normal guinea pigs and the preparation of smears for the microscopic study of its fluid content. There were moments of real excitement when the examination of the first slides revealed an impressive wealth of diverse cell forms and a sequence of distinctive cytologic patterns by which the vaginal, as well as the uterine and ovarian cyclic morphologic changes and the exact time of ovulation could be accurately determined.

One thing we should never forget is the debt we owe to our predecessors for their priceless treasury of thought, which provided the roots for our inspirations and investigative directives. As Bishop wrote in 1844: "An important and comprehensive discovery has almost never seen the light of day suddenly; generally, the elements of the discovery are contributed from various directions, making the unfolding of the truth possible."

The elements that made possible the discovery of the vaginal cell cycle in the guinea pig almost 40 years ago certainly had

their roots in countless contributions to knowledge, chiefly from the last century, in anatomy, histology, reproductive physiology, biochemistry and cellular pathology.

One may thus look at the advancement of science in any particular field as an endless chain of ideas intimately linked with observations always alternating and constantly giving birth one to another.

With this in mind one wonders whether all of our activities—scientific and otherwise—are actually governed by our own free will alone or also by forces lying beyond our grasp and control. No matter what the answer may be, one who has devoted his life's work to the furtherance of scientific knowledge may draw satisfaction from the conviction that he has within his own ability contributed to the enrichment of life and the alleviation of human suffering.

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PROCEEDINGS TEXAS BRANCH, SOCIETY OF AMERICAN BACTERIOLOGISTS

1955 Meeting

Radiation Resistance and Violacein Production. William R. Romig, T. P. O'Barr, and Orville Wyss, Department of Bacteriology, The University of Texas, Austin.

Chromobacterium violaceum is unusually sensitive to inactivation by ultraviolet light and highly susceptible to photoreactivation. Berger showed that anaerobically grown pigmentless cells were more resistant to killing than aerobically grown pigmented cells but since it is now known that most bacteria are more resistant to ultraviolet under anaerobic conditions, the role of the pigment independent of anaerobiosis was examined. Alcoholic solutions of the pigment, added to *Escherichia coli* B/r which were then irradiated, did not appear to affect the survival of the organisms, nor were significant effects on photoreactivation observed in such experiments.

Modified degrees of pigmentation were obtained by selecting mutants of *C. violaceum* and by devising various synthetic media. In neither case was ultraviolet sensitivity correlated with pigmentation. It was concluded that this organism's sensitivity to radiation is not dependent on pigmentation *per se*.

Calcium Content of Spores of Bacillus cereus var. mycoides in Relation to Heat Resistance and Content of Dipicolinic Acid. J. J. Perry and J. W. Foster, Department of Bacteriology, The University of Texas, Austin.

Well-washed spores produced endotrophically from vegetative cells harvested from glucose-salts (M-9) medium contained 0.034 per cent calcium; the vegetative cell contained 0.024 per cent. Spores formed in glucose-salts growth medium supplemented with 0.5 per cent yeast extract contained 0.179 per cent calcium, and the vegetative cells from this medium contained only one-half that level of calcium. Thus, in contrast to the data of Curran *et al.* (*J. Bact.*, 45: 485, 1943) spores in these experiments were not uniquely enriched in calcium as compared to the corresponding vegetative cells. The higher content of calcium in spores from yeast medium could be a non-specific absorption.

Growth medium and endotrophic spores contained respectively only 0.14 and 0.021 milli-equivalents of calcium per milli-equivalent of dipicolinic acid contained in the spores, hence the latter compound does not exist in the spores entirely in the form of calcium salt.

Evaluation of Antiseptic Toxicity in Skin Tissue Culture. Tom Wynne, C. M. Pomerat, and F. B. Engley, Jr., The University of Texas Medical Branch, Galveston.

A test procedure has been developed to answer a great need in the field of antiseptics and disinfectants. Previously, toxicity determinations have been carried out in animals, on chick heart or on leukocytes. The procedure described uses human skin tissue cultures of pure epithelial growth rather than fibroblasts. The chemicals are added to the skin in tissue culture and observed for their toxicity for the skin. Observations reveal marked differences in the ability of various chemicals to prevent the outgrowth of the skin. Comparisons have been made with particular reference to skin antiseptics such as the mercurials which were found to be very toxic. The test is also adaptable for sulfonamides, antibiotics and other chemotherapeutic agents.

Paradoxical Viability of Anthrax Spores. R. P. Williams and R. Miller, Department of Microbiology, Baylor University College of Medicine, Houston 25, Texas.

Pasteurized anthrax spores harvested from casein-yeast extract medium after 30 hours incubation at 37° C. showed only 10 to 50 per cent the amount of growth as spores harvested after 90 hours when the two groups of spores were transferred to synthetic medium. Results of our experiments showed that there was no difference in the growth of 30- and 90-hour spores if the same inoculum size of spores was used. The paradoxical growth observed in preliminary experiments was due to the size of the spore inoculum. In addition our results showed that synthetic medium was not adequate for viability of all spores in an inoculum. Only 1 to 2 per cent of spores viable on casein-yeast extract medium were also viable on synthetic medium. Also more pasteurization resistant spores were present after 90 hours incubation on casein-yeast extract medium than after 30 hours incubation.

Phenyl Ethyl Alcohol as a Topical Anti-Microbial Agent. Joseph A. Bass, Truman G. Blocker, Jr., Charles R. Mowery, John W. Kolaja, Jr.. The University of Texas Medical Branch, Galveston.

The use of phenyl ethyl alcohol as an inhibitory agent in isolation media suggested its application as a topical antimicrobial agent in burns, wounds, and ulcers. In the present study, it has been tested on 20 patients having moderately to grossly infected wounds. Used as soaks, 1:60 and 1.120 dilutions in physiologic saline have proved as effective as Neomycin soaks in reducing the extent of infection. When combined with Neomycin, effectiveness of the soaks appears to be enhanced. Skin graft "takes" have generally been very satisfactory following such treatment.

No markedly antagonistic effects have been noted *in vitro* when phenyl ethyl alcohol was tested with 11 of the major antibiotics. The use of related compounds such as phenyl carbinol is also being investigated.

Further Evidence of Germination at 75° C. of Spores of Mesophilic Clostridia. L. I. Galyen and E. Staten Wynne, Section of Experimental Pathology, The University of Texas. M. D. Anderson Hospital and Tumor Institute, Houston.

When spores of *C. botulinum* 62A, *C. botulinum* 115B, *C. feseri*, *C. perfringens*, and putrefactive anaerobe No. 3679 were incubated at 75° C. in autoclaved buffered glucose, a rapid decrease in the number of recoverable spores was observed. That germination occurred was supported by the following observations:

(1) Sodium oleate, a known inhibitor of germination, also *increased* the numbers of recoverable spores in this system. (2) lowering the pH of the medium from 7.5 to 5.0 increased recoverable spores, contrary to expectation with thermal destruction of spores. (3) Addition of the purine analogs 2,6-diaminopurine, 6-chloropurine, 8-azaguanine and 6-mercaptopurine increased recoverable spores. These increases in recoverable spores were interpreted as resulting from inhibition of germination. There was also an increase in the number of spores failing to retain the primary stain used in the Wynne spore stain (*J. Inf. Dis.*, 83: 243–249, 1948).

A Bacteriophage for Azotobacter. Douglas S. Kellogg, Jr., and Orville Wyss, Department of Bacteriology, The University of Texas, Austin.

We have isolated from soil an agent which forms plaques on *Azotobacter vinelandi*. It does not form plaques on the unencapsulated forms of *A. vinelandi* designated as "B" nor on the nonpigmented strains which are generally labeled *A. agile*. Three strains of *A. chroococcum* tested were not found to be sensitive. Complete cultural lysis has never occurred since a rough nonencapsulated strain always arises and appears to be resistant. The phage has been stored under refrigeration in its cultivation broth for over a year without perceptible loss in titer. It is relatively sensitive to centrifugation in that centrifugation for thirty minutes at 10,000 times gravity in the refrigerated Spinco centrifuge destroys the infectivity of the particles and produces considerable cytological damage. Measurements by electron microscopy show a head 101 by 93 millimicrons and a tail 150 millimicrons in length.

Some Observations on Bacterial Contamination in a Burn Service. Jose A. Rivera, Anthony Contreras, and Curtis P. Artz, Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas.

These studies reveal the possible sources of bacterial contamination in the wounds of burned patients. The bacteriologic findings on these patients were compared with findings on doctors, nurses, other ward personnel, and also with bacteria cultured from air samples of the wards and operating rooms. Routine and special culture methods included antibiotic sensitivity tests on each one of the organisms isolated.

The predominating organisms cultured from the wounds of the burned patients, in their order of frequency, were: *Micrococcus pyogenes*, *Pseudomonas*, and *Proteus*. These organisms were also isolated from the blood stream of burned patients who had developed septicemia.

The predominant organism cultured from the ward personnel was *Micrococcus*, coagulase-negative, and not *Micrococcus pyogenes*, coagulase-positive.

The predominant organism cultured from the air samples was *Micrococcus*, coagulase-negative.

It was concluded that bacteria contamination through the air, by ward personnel, or cross-infections by patients, was at a minimum.

Some Microbial and Chemical Conditions Associated with Phymatotrichum Root Rot Infested Blackland Soils. Ralph J. Hervey, Texas Agricultural Experiment Station, Temple.

Organic residues and microbial antagonism are apparently important factors in controlling Texas root rot, caused by the fungus *Phymatotrichum omnivorum.*

Houston black clay samples, at 6 inches profile intervals to 24 inches, were collected from cotton fields with high, intermediate and low disease incidence and low, medium and high yields. High disease-low yield plots averaged 38 per cent dead plants and yielded 270 pounds of cotton; low disease-high yield plots averaged 1 per cent disease and yielded 659 pounds.

Fungus, actinomycete and total bacterial populations, organic matter, total nitrogen and nitrates of the low disease-high yield soils were significantly greater than those of the high disease-low yield soils. Greatest microbial differences occurred in the near-surface zones. Populations of the better soils within the 24-inch profile, averaged 25 to 50 per cent higher while organic matter, total nitrogen and nitrates averaged 40 to 50 per cent higher.

The Effect of Food Intake on Microbiologically Estimated Amino Acids in Unhydrolyzed Human Plasma. Cora L. Gott and E. Staten Wynne, Section of Experimental Pathology, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston.

Plasma drawn from volunteers before, and 1 and 3 hours after eating breakfast was assayed microbiologically for 6 amino acids by the method of Henderson and Snell (*Jour. Biol. Chem.*, 172: 15–29, 1948). Levels of tryptophane, glycine, isoleucine, and tyrosine were not appreciably increased after eating. With histidine, only 1 of 13 tests showed a significant increase ($p<0.01$) after eating, but this increase was demonstrable in only one of 3 tests on the same individual. Of 11 experiments on 8 subjects, arginine was increased in 10, with 4 significant at the level $p=0.01$. Arginine levels exhibited considerable variation among individuals and in the same individual tested at different times,

but analysis of variance showed the trend toward increase on eating to be highly significant ($p=0.001$).

Pantothenic Acid Reversal of 6-Mercaptopurine Inhibition of Aerobacter Aerogenes and Potentiation of the Inhibition by Other Inhibitors of Pantothenic Acid Biosynthesis. Thomas Stim, Bennie Arnwine, and J. W. Foster, Department of Bacteriology, The University of Texas, Austin.

Pantothenic acid and pantoic acid reversed the inhibition caused by 6-mercaptopurine in the spread plate procedure employing M-9 agar medium (Foster and Pittillo, *J. Bact.*, 66: 478–486, 1953). From 0.5 to 1.0 mg. 6-mercaptopurine was required to prevent growth for approximately 24 hours, but by 36–48 hours growth had occurred. Uninhibited controls showed good growth in approximately 16 hours. Under these conditions 6-mercaptopurine presumably inhibits the synthesis of pantoic acid moiety of pantothenic acid (Pittillo and Foster, *J. Bact.*, 67: 53–57, 1954) for 24 hours. Other substances known to inhibit the synthesis of pantothenic acid, *e.g.*, D-serine and also salycelic acid, display a strong synergistic action in inhibiting the bacteria when used with 6-mercaptopurine. The synergism is explained by the fact that the two inhibitors block the synthesis of pantothenic acid simultaneously at two different points.

Studies on Recently Isolated Strains of Proteus from Burned Patients. Jose A. Rivera, Russell Brame, and Ruth Latta, Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas.

Proteus organisms have become predominant and persistent in infections of burned patients. In some instances they have invaded the blood stream. Five patients who died last year of septicemia had positive blood cultures showing *Proteus*.

During a five-year period, *Proteus* have been 100 per cent resistent to penicillin, and 37.5 per cent resistant to Chloromycetin. Resistance to other antibiotics has also been high in percentage.

In a series of cultures from 19 patients, 16 were *Proteus mirabilis* and 3 were *Proteus vulgaris*. In another larger series of 186 patients, 175 ,93.8 per cent) were *Proteus mirabilis*, and 11 (6.2 per cent) were *P. vulgaris*.

Chloromycetin sensitivity tests carried out on 170 strains showed that 96 (56.5 per cent) were sensitive, while 74 (43.5

per cent) were resistant. Of the 96 sensitive strains, 83 were *P. mirabilis*, and 13 *P. vulgaris*. All the 74 Chloromycetin-resistant strains were *P. mirabilis*.

Human Tissues Used for Virus Experiments. Helene Wallace Toolan, Sloan Kettering Institute for Cancer Research, New York City, and Surgical Research Unit, Brooke Army Medical Center, Fort Sam Houston, Texas.

For many years, research workers have attempted to grow human tissues, both normal and malignant, outside the human body. It is now possible to obtain large volumes of human tissue (one tumor is being produced at the rate of 3 pounds per week) for experimental use by growing them in rats and hamsters treated with cortisone and/or x-radiation. Once it has been grown in such animals, the human material will also proliferate with ease in eggs and tissue culture. The advantage of having viable human tissues outside the human body is obvious. As an example, one of the studies now being carried out is a screening program with viruses possibly effective in destroying cancer cells but too generally dangerous to use, in their present form, directly on the patient. Should one of them prove effective on these human tumors in the experimental animal, egg or tissue culture, it can then presumably be attenuated for patient use.

Influence of Sporulation Upon Streptomycin Resistance of Bacillus Globigii. Russell G. Weichlein and Roy B. Mefferd, Jr., Southwest Foundation for Research and Education, and Trinity University, San Antonio, Texas.

The nature of streptomycin resistance remains unknown. Were resistance the result of adaptive enzyme mechanisms, it should decline following repeated sporulation, since the quantities of such enzymes would decrease in the absence of further stimulation.

Sixty-four subcultures which had never been exposed to streptomycin and thirty which had been exposed one time only, were passed through six sporulation cycles. At each cycle aliquots of each culture were assayed for streptomycin resistance before and immediately after heating, and following a two-hour germination.

Resistance passes through the spore stage, and in cultures never exposed to streptomycin the populations were generally

stable in broth culture from passage to passage. Exposure to streptomycin resulted in populations of high resistance, and erratic shifts in these from cycle to cycle.

The evidence, although not conclusive (since some enzyme molecules undoubtedly pass sporulation) is highly indicative of genetic control rather than of an adaptive enzyme mechanism.

Factors Influencing Chromogenesis in Micrococcus Pyogenes. James A. Johnston, Department of Bacteriology and Parasitology, The University of Texas Medical Branch, Galveston.

Pigment formation in *Micrococcus pyogenes* shows a high correlation with pathogenesis. It is desirable, therefore, for diagnostic purposes to cultivate the organism in a manner which is conducive to rapid pigment formation, and it was early recognized that the addition of milk to culture media promotes the formation of pigment. This report deals with the isolation of the pigment-stimulating factor from milk.

Using a Difco Proteose Peptone No. 3 agar, which alone does not support pigment formation, as a basal medium, it was found that the pigment factor was associated with the casein fraction of milk. Subsequent purification of the casein revealed that the metal calcium was in fact the pigment factor. The effect of calcium was antagonized by magnesium. A variety of other cations were tested and found to be inactive in pigment formation under conditions of the study. Approximately one hundred pigmented strains of *M. pyogenes* were examined, and calcium stimulated the production of pigment in more than 90 per cent of the strains.

Tissue Culture and Gel Diffusion Techniques in the Demonstration of Tumor Immunity Factors. Paul Mazel and Roy B. Mefferd, Jr., Southwest Foundation for Research and Education, and Trinity University, San Antonio, Texas.

We have observed increased resistance, or enhancement in Sherman rats, depending on dosages, with living tumor tissue antigens. Sera from control, enhanced, resistant and extracts of spleens and livers was subjected to serological and agar-diffusion to determine if antigen-antibody mechanisms were demonstrable. The effects of each serum upon the fibrosarcoma in tissue culture was determined.

Classical serological techniques were negative. Agar-diffusion tests showed no identities between extracts. A number of identities between the sera and the tumor antigens, and a partial identity between negative and enhanced sera was observed. Negative serum was inhibitory to the fibrosarcoma in tissue culture at high dilutions while a 1:10 dilution of other sera were not.

Resistant sera contains an antibody-like substance not present in normal or enhanced sera which is demonstrable only by biological or other sensitive techniques. Enhanced serum showed the presence of a factor stimulatory to cell growth.

ELECTRON MICROSCOPIC STUDIES OF THE ROSEOLA INFANTUM (EXANTHEM SUBITUM) VIRUS*

REGINALD L. REAGAN, SING CHEN CHANG, SYLVAN E. MOOLTEN, ELLEN CLARK AND ARTHUR L. BRUECKNER

Roseola infantum may be defined as a mildly contagious, acute illness confined to infants and young children. It is characterized by sudden onset and by fever, from 3 to 5 days, which terminated on or just before the appearance of a transient fine rash upon the neck, trunk, and upper extremities (Rake *et al.*, 1952).

Zahorsky (1910) was the first to describe the disease as a distinct entity. Although known for over a century, the disease had formerly been overlooked due to confusion of it with other exanthemata.

The disease is most prevalent in children under 4 years, however, Cutts (1938), and Clements (1945) described a typical case in a 31-year-old woman. The rash is mascular and fine in typical cases but may be maculo-papular. The rash fades rapidly, desquamation is rare and pigmentation does not occur.

The causative agent is believed to be a virus. Only negative results have occurred upon attempts to cultivate bacteria from the blood at various stages of the disease. Kempe *et al.* (1950) were able to transmit the disease from one infant to another by means of intravenous inoculation of serum collected on the third day of the febrile period. Roseola infantum was also transmitted to monkeys, both from the donor and from the recipient by means of serum or throat washings taken on the third day of fever. The monkeys developed fever and leukopenia, but neither exanthem nor enanthem. The incubation period was 4 or 5 days. The febrile disease was passaged to other monkeys by means of serum taken on the second day of fever and inoculated either untreated or after filtration.

Materials and Methods

Heparinized venous blood was collected from a patient (age 18 months) with Roseola infantum at the height of the fever and upon appearance of the rash. The sample was supplied through the courtesy of Dr. Sylvan E. Moolten and Dr. L. M. Krafchik of the city of New Brunswick, N. J., of the St. Peter's

* From the Virus Department, Live Stock Sanitary Service Laboratory, University of Maryland, College Park, Maryland, and St. Peter's General Hospital, New Brunswick, New Jersey.

General Hospital, New Brunswick, New Jersey. The patient was bled from the marginal vein, and the heparinized blood was shipped in dry-ice for initiating the experiment at the University of Maryland.

Monkeys (Macaca rhesus) weighing from 2 to 3 lbs. were used throughout the experiment. Each animal was housed for the period of the experiment in individual cages and isolation precautions were maintained in handling the animals. Rectal temperatures were taken twice daily but no blood was drawn for blood leucocytic examination. The animals were caught in such a manner as to minimize exertion and excitement and then were allowed to relax before the temperatures were taken. A diet consisting of milk, bananas, oranges, bread, and greens was provided throughout.

Two monkeys, No. 1 and No. 2, were lightly anesthetized with ether and were exposed intradermally with 3 ml. of the heparinized blood from the Roseola infantum case stated above. Two more monkeys, No. 3 and No. 4, were injected with normal heparinized blood in the same manner. Six days post inoculation the rectal temperature of monkey No. 1 reached 104.1° F. and the animal was bled from the heart for 10 ml. of blood. On the same day, monkey No. 2 had recorded a rectal temperature of 103.9° F. and was similarly bled. This particular monkey died suddenly 10 days post inoculation and autopsy revealed an enlarged spleen but otherwise apparently healthy organs and tissues. Monkeys No. 3 and No. 4 remained normal on the 6th day after inoculation, monkey No. 3 was bled from the heart for 10 ml. of blood which was used as a normal control.

The pooled infected blood from monkeys No. 1 and No. 2 and the normal blood from monkey No. 3 was kept at —4° C. for 1 hour. The blood specimens were then centrifuged for 1 hour at 2,000 r.p.m. The serums were then removed from each and then subjected to centrifugation under refrigeration in a Spinco ultracentrifuge for 2 hours at 44,700 r.p.m. The temperature of the refrigerated outer jacket stayed constant at —8° C. during the centrifugation. After the 2-hour centrifugation period, the supernatants from the specimens were discarded, and the sediment from each specimen was resuspended in physiological saline. Several drops of each suspension were placed on several parlodion film supports which had been prepared 48 hours previously. After all excess fluid had been removed with small capillary pipettes, the films were dried and shadowed with chromium (Williams and Wyckoff, 1946) at arc tangent 2/10 and examined under the R.C.A. electron microscope, type E.M.U.

The balance of the material of the infected serum was injected intradermally into 2 susceptible monkeys (No. 3 and No. 4) and into monkey No. 1 which was now immunized. Rectal temperatures were taken twice daily of the 3 animals and 5 days post inoculation monkeys No. 3 and No. 4 had recorded temperatures of 104.2° F. Monkey No. 1 (immunized animal) remained normal throughout the observation period.

Results

Upon electron microscopic examination of the concentrated suspension from the infected serum, uniform virus-like particles were observed. They were present in the majority of the 2,000

microscopic fields examined. Figures I and II are electron micrographs showing these virus-like particles. The majority of these particles are spherical in shape and measure 100-110 mμ by direct measurement. Irregular contours may be seen on occasional particles, but they may be due to the hypertonicity of the suspending fluid during the drying process.

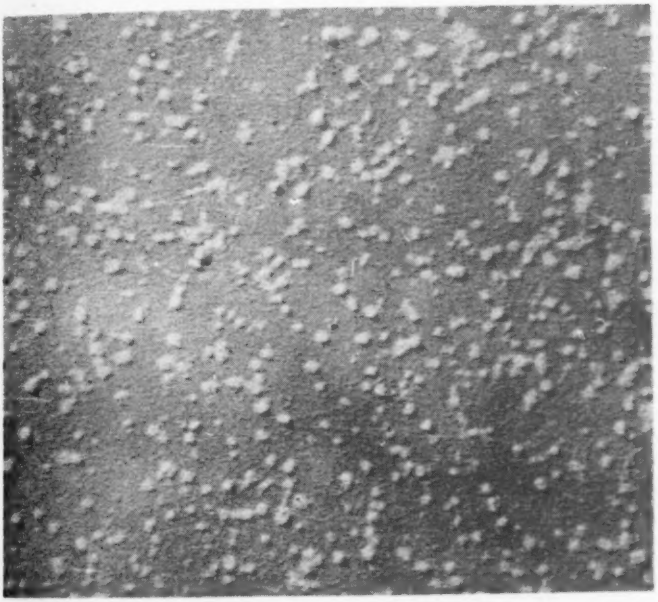

FIG. I. Electron micrograph of the Roseola infantum virus. Shadowed with chromium at arc tangent 2/10. x 72,000.

No virus-like particles were observed in the concentrated normal serum in approximately 2,000 fields examined by electron microscopy.

Summary. Studies by electron microscopy of serum of monkeys infected with the virus of roseola infantum show the virus to be spherical, with occasional particles having irregular contours. The particles have a diameter of 100-110 mμ by direct measurement. These bodies could not be demonstrated in normal monkey serum subjected to the same procedure of concentration and electron microscopic examination. These bodies resemble, to some

degree, the virus-like bodies of measles described by Reagan *et al.* (1932). This virus in the concentrated material was infectious for susceptible monkeys but had no effect on a monkey previously imunized against roseola infantum.

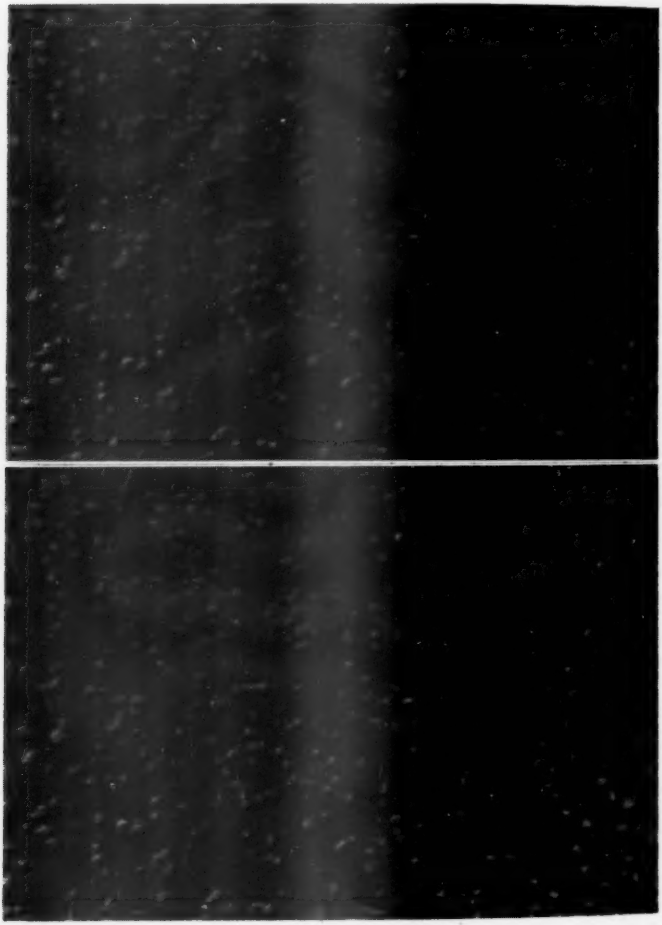

FIG. 2. Electron micrograph of the Roseola infantum virus. Shadowed with chromium at art tangent 2/10. x 36,000.

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ELECTRON MICROGRAPHS OF ERYTHROCYTES FROM SWISS ALBINO MICE INFECTED WITH ZIKA VIRUS*

REGINALD L. REAGAN, SING CHEN CHANG, A. L. BRUECKNER

The Zika virus (Smithburn and Haddow) was furnished by the American Type Culture Collection in Washington, D. C. (Public Health permit was obtained to work with this virus.) The Zika virus was obtained by the above institution from Dr. K. C. Smithburn of the Rockefeller Foundation Laboratories in New York. This virus was encountered by these workers in field investigation to discover the insects responsible for the transmission of yellow fever in extra-human cycles of natural infection in Uganda Protectorate, British East Africa. Mosquitoes were captured in selected localities and classified. Suspension of these mosquitoes were inoculated into susceptible laboratory animals (mice and rhesus monkeys), to test the presence of virus.

Materials and Methods

Lyophilized mouse brain of the 146th intracerebral mouse passage was diluted to a 10 per cent suspension with sterile distilled water. Each of ten 3-week-old Swiss albino mice was inoculated intracerebrally with 0.03 cc. of this suspension. On the 6th day after inoculation, the mice showed symptoms of central nervous system involvement. When symptoms appeared, the mice were sacrificed, and the brain was removed aseptically, pooled, ground and diluted to a 10 per cent suspension. This was injected intracerebrally into six unvaccinated mice and into six mice which previously had been immunized intramuscularly with a high dilution of Zika virus. After six days, the unvaccinated mice showed symptoms of nervous system involvement, while the immunized group showed no nervous symptoms. This confirmed the virus suspension to contain Zika virus and for the present experiment this was used as the inoculum which titrated 10^{-5} intracerebrally in Swiss albino mice. The present experiment was conducted in the following manner. Thirty-two 21-day-old, healthy Swiss albino mice (Webster and Dawson, 1935), were divided into two groups of sixteen mice each. One group was designated as the control group and the other as the test group. One-tenth cc. of a 10 per cent suspension of the above described inoculum was administered the mice by rectal instillation. For the rectal instillation, the end of a 22 gauge needle was filed off, rough ends smothed down, and the tip lubricated with vaseline before being inserted into the lumen of the rectum, and the control group was administered 0.1 cc. of a 10 per cent normal mouse brain suspension like-

* From the Virus Laboratory, Live Stock Sanitary Service, University of Maryland, College Park, Maryland. Received for publication April 28, 1955.

wise. The mice of each group were further divided into eight groups of two mice each. Two mice for the test group and two mice for the control group were bled from the heart two hours post-inoculation. Approximately 0.06 cc. of blood was drawn from each animal. The blood taken from two animals in the test group was pooled and added to 1 cc. of heparinized physiological saline. The blood from the two animals of the control group was treated in the same manner. The cell suspension from each group was then employed in electron microscopic studies. The same procedure was carried out on trythrocyte suspensions taken from mice of the test and control groups at time intervals of 16, 24, 40, 48, 72, 96 and 120 hours post-inoculation. Only animals from the test group at the 120-hour interval exhibited symptoms of central nervous system involvement such as that found in mice infected intracerebrally with this virus. The mice from the control group showed no symptoms of nervous system involvement.

Each specimen was prepared for electron micrograph examination by placing several drops of the erythrocyte-heparinized saline suspension in distilled water for ten seconds (to remove hemoglobin so that the electron beam would penetrate the erythrocyte). This suspension was then placed on parlodion film supports which had been prepared two weeks previously. The films were dried and shadowed with chromium (Williams and Wyckoff, 1946) at art tangent 2/12 and examined under the R.C.A. electron microscope, type E.M.U.

In order to determine the infectivity of the erythrocytes obtained from animals of the test group thirty-two mice were divided into eight groups of four Swiss mice each, and mice from each group were inoculated intracerebrally with 0.03 cc. of an erythrocyte suspension at a designated time interval. In order to determine the presence of any toxic material in the diluent (heparinized physiological saline), ach of four Swiss mice were inoculated intracerebrally with 0.03 cc. of an erythrocyte heparinized saline suspension from the control group. The erythrocyte specimen was taken from mice of the 72-hour group. After inoculation, all mice were observed twice daily over a 21-day observation period.

Results

Upon examination by electron microscopy of the erythrocytes taken at designated time intervals from the control group, no virus-like particles could be seen. However, upon examination, the erythrocyte suspension taken from the infected Swiss albino mice, a few virus particles were seen on the surface of the erythrocytes obtained at the time interval of 16 hours as shown in figure I. These virus-like particles had the same shape and size as those demonstrated by electron microscopy of the Zika virus from infected mouse brains (Reagan and Brueckner, 1953). Virus-like particles could not be demonstrated in the interval bleedings of the other test or control groups. A representative micrograph of the control group (72 hour) is presented in fig-

ure II. Table 1 shows the infectivity of erythrocyte specimens from the test group injected introcerebrally into Swiss mice. These mice succumbing to the disease, developed symptoms of central nervous system involvement within 6 to 10 days after inoculation. All mice inoculated from the control group (72 hour) remained normal throughout the 21-day observation period.

FIG. I. Erythrocyte from mouse infected intrarectally with Zika Virus. Time interval—16 hours. Shadowed with chromium at arc tangent 2/12 x 32,000. Virus-like particles indicated by arrows.

Summary. From the results obtained in this experiment, it was apparent that the virus was present in the blood stream from 2 to 24 hours. Electron micrographs of the virus-like particles showed the virus to be in or on the erythrocyte only at the 16 hour interval bleeding. This was the only bleeding infective for Swiss mice inoculated intracerebrally. Virus-like particles could not be demonstrated in the other bleedings of the test or control group.

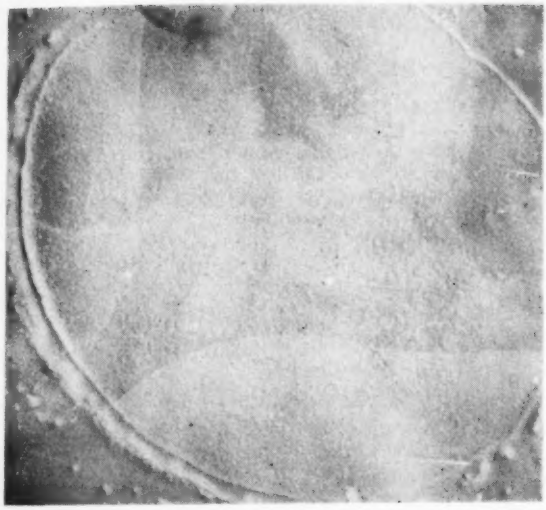

FIG. II. Erythrocyte from mouse exposed intrarectally with normal mouse brain. Time interval—72 hours. Control representative from all control groups. Shadowed with chromium at arc tangent 2]12 x 32,000.

TABLE 1

Response of Swiss Albino Mice Infected Intracerebrally with Erythrocytes from Swiss Albino Mice Infected Intrarectally with Zika Virus

Bleeding Intervals (Hours)	No. of Mice Inoculated with Erythrocyte	Mice Showing Symptoms	Min. to Max. Inoculation Period (Days)
2	6	0	.
16	6	6	6–10
24	6	0	.
40	6	0	.
48	6	0	.
72	6	0	.
96	6	0	.
120	6	0	.

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THE NUCLEUS LOCUS COERULEUS (DORSOLATERALIS TEGMENTI)

GLENN V. RUSSELL*

I. Introduction

The nucleus locus coeruleus (dorsalateralis tegmenti) of man and the higher primates is, as its name implies, a darkly colored region in the dorsolateral portion of the rostral metencephalic and caudal mesencephalic tegmentum. Despite its large size in most mammals it has received only scant attention. It has been considered by different authors as (1) related to the trigeminal complex and thus a part of the somesthetic mechanism for the head, (2) a portion of the mechanism of visceral sensibility, and (3) a part of the regulatory mechanism for respiration.

The ambiguity surrounding this large nucleus suggested the need for a review of the literature on the subject and for a re-evaluation of the comparative anatomy and connections of this nucleus in a wide series of mammalian forms. A collateral investigation, already reported (Johnson and Russell, 1952), demonstrated that this nucleus subserves a respiratory function, at least in part, and a descending lateral tegmento-reticular tract from the nucleus locus coeruleus to the medullary reticular formation dorsal and dorsomedial to the inferior olivary complex was described in the cat. That suprasegmental influence may be exerted through the nucleus locus coeruleus upon general visceral motor activity, including the specifically demonstrated effect upon respiration, was suggested.

II. Review of the Literature

A. The Nucleus Locus Coeruleus. In 1809 Reil described, but did not name, a depression in the anterior floor of the fourth ventricle of man where a streak of blue-black substance shown through. He described a stria of fibers which appeared to course from this region across the floor of the ventricle, and remarked upon the great vascularity of the pigmented region itself. This

* From the Department of Anatomy, The University of Texas Medical Branch, Galveston. A large part of this communication is drawn from a thesis presented to the faculty of Cornell University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

pigmented area was redescribed by the brothers Joseph and Karl Wenzel (1811) and named by them the locus coeruleus. Unfortunately they included in their work an incorrectly labeled plate (Tab. X, Fig. 4). Arnold (1838, 1851) renamed the region the substantia ferruginea but, apparently mislead by the Wenzels' figure, mislocated it in the anterior superior fovea of the ventricular floor. The error went uncorrected until 1872 when Meynert stated: "In the rostral floor of the fourth ventricle, lateral to the eminentia teretes, is a depression, the fossa coerulea, dark because of the pigmented cells of the substantia ferruginea beneath." He described the progressive pigmentation of the cells of this nucleus from the infant in which no pigment was observed to the adult pigmented condition. Meynert suggested that the nucleus gave rise to the middle portion of the mesencephalic root of the trigeminal nerve and possibly to a part of the inner portion of that root.

Huguenin (1875) reported identifying the axons which arise from the cells of the nucleus locus coeruleus but failed to suggest a destination for them. He measured the size of the cells of the nucleus: ". . . messen in sagittal gestellten grössten Durchmesser 60–70 microns, senkrecht darauf 20–30 microns." Forel (1877) confirmed Meynert's observations on the progressive pigmentation of the cells of the substantia ferruginea, and suggested that a homologous nucleus, although unpigmented, existed in the brainstem of subprimate mammals. He believed that the cells of the nucleus being without pigment would be difficult to differentiate from the cells of the surrounding central gray matter. Forel denied Meynert's assertion that this nucleus gives fibers to the mesencephalic root of the trigeminal nerve stating that this relationship is apparent rather than real. Schwalbe (1881) concurred with Forel, stating: ". . . der substantia ferruginea, . . . hat aber mit dem Ursprung des Trigeminus nichts zu thun." Bechterew (1887) was also of this opinion. Mendel (1888) believed that some of the fibers of the mesencephalic root of the trigeminal nerve were derived from cells of the nucleus locus coeruleus.

Homén (1890), in reporting a description of the brainstem of a case of hemiatrophia facialis, found only slight, if any, differences between the nuclei locus coeruleus on the two sides. Comparison of the fibers from the nucleus, fibers which he thought formed a poorly defined bundle in the median longitudinal fasciculus, showed no appreciable differences on the two sides.

Obersteiner (1890) described the nucleus locus coeruleus as consisting of cells easily recognizable as belonging to the visceral column of Clarke, and asserted that such an intermittent column of neurons could only be visceral in nature. He described a bundle of fibers from the nucleus locus coeruleus which passed medially beneath the floor of the fourth ventricle, crossed the midline piercing the median longitudinal fasciculus, and turned caudally to join the motor root of the trigeminal nerve of the opposite side. He also reported fibers from the nucleus locus coeruleus which joined the mesencephalic and motor roots of the trigeminal nerve of the same side. Gudden (1891) confirmed the crossed bundle described by Obersteiner and described it as being 0.8 mm. thick.

Poniatowsky (1892) reported that the nucleus locus coeruleus gave a crossed bundle of fibers to the sensory root of the trigeminal nerve, while Golgi (1893) much in error and apparently confused, mislocated the nucleus and described it as an aggregate of monopolar cells giving rise to the trochlear nerve. Held (1893) included the nucleus locus coeruleus among the principal sensory nuclei of the trigeminal nerve. Hösel (1893) reported no apparent change in the nucleus locus coeruleus, or in the mesencephalic or motor nuclei of the trigeminal nerve, in a case in which the superior sensory nucleus of the trigeminal nerve had been completely destroyed.

Cramer (1894) concurred with Meynert and Obersteiner in considering the nucleus locus coeruleus as one of the nuclei of origin for the mesencephalic root of the trigeminal nerve. Lugaro (1894), however, failed to even mention the nucleus in his discussion of this root. Huguenin (1895) and Kölliker (1896) simply considered the nucleus locus coeruleus as a part of the mesencephalic nucleus of the trigeminal nerve. Kljatschkin (1897) reported no retrograde changes in the nucleus locus coeruleus of either side following unilateral trigeminal nerve root section in the cat, however; nor was degeneration observed within the trigeminal complex after destruction of the nucleus locus coeruleus.

Kuré (1899) described two types of cells in the nucleus locus coeruleus. The larger cells he believed to be analagous with the cells of the mesencephalic nucleus of the trigeminal nerve and he reported that they underwent chromatolysis following section of that nerve. The smaller cells were not thus affected. Terter-

janz (1899) described the axons from the cells of the nucleus locus coeruleus as crossing the raphe above the median longitudinal fasciculus and turning rostrally and laterally to join the mesencephalic root of the trigeminal nerve.

Kohnstamm (1900) described the group of multipolar neurons in the region of the mesencephalic root and nucleus of the trigeminal nerve (the multipolar Vth cells of Kölliker: Fig. 503, Gewebelehre, 6th Edit., 2: 1896) as the nucleus spinalis tecti intratrigeminalis. He cited Kuré's description of these cells as a part of the nucleus locus coeruleus, but stated that his nucleus was not the homolog of the nucleus locus coeruleus and that it gave rise to no descending axons.

Bickel (1901) observed the nucleus locus coeruleus and referred to Meynert, Obersteiner, Cramer, and Terterjanz. Edinger (1904) did not distinguish between the nucleus locus coeruleus and the mesencephalic nucleus of the trigeminal nerve. He stated: ". . . scattered ganglion cells give rise to the radix mesencephalica nervi trigemini. These cells continue downward always in the lateral wall of the canal, and one can detect a more considerable collection of them through the thin ventricular ependyma, forming a dark bunch on either side at the beginning of the fourth ventricle. It is there called the locus coeruleus."

Lewandowsky (1904) sought the origin of Probst's tract among the polygonal cells in the region of the mesencephalic root of the trigeminal nerve, the nucleus spinalis tecti intratrigeminalis of Kohnstamm. However, Kohnstamm (1905) redescribed his nucleus and placed it somewhat more rostrally in the brainstem and suggested that it was a part of the optic reflex system and sent fibers into the spinal cord via the median longitudinal fasciculus of the opposite side. Lewandowsky had suggested that Kohnstamm's nucleus spinalis tecti intratrigeminalis was actually the nucleus locus coeruleus and that it was the nucleus of origin for Probst's tract. This tract, he proposed, influenced the medullary respiratory centers and thus the nucleus locus coeruleus belonged to the regulatory mechanism for respiration. Kohnstamm denied these suggestions very positively.

Calligaris (1908) discussed the pigmentation of the cells of the nucleus locus coeruleus and gave an extensive bibliography on the subject of pigmentation in nerve cells, but he was unable to suggest the purpose or function either of the pigment or of the cells. Kohnstamm and Quensel (1908) reported that the cells of

the real nucleus locus coeruleus are vesicular in shape and are intermingled with those of the mesencephalic nucleus of the trigeminal nerve. The fibers of this nucleus join the motor root of the trigeminal nerve according to them.

Cajal (1909) described the nucleus locus coeruleus as being composed of fusiform or pyramidal cells lying primarily on the internal surface of the mesencephalic root of the trigeminal nerve. He stated that he had never observed axons from these cells joining those of the mesencephalic root of the trigeminal nerve. He described the dendritic arborization of these cells as being very extensive. It is enmeshed in a very complicated neuropil which extends throughout the nucleus locus coeruleus and which is continuous with the neuropil of the mesencephalic nucleus of the trigeminal nerve. This neuropil, Cajal thought, received a large number of fibers from the superior vestibular nucleus. The axons of the cells of the nucleus locus coeruleus were described by Cajal as very thin, much thinner than those of the cells of the mesencephalic nucleus of the trigeminal nerve, and directed medially into the central gray matter. He suggested that it was possible that this nucleus was a sensory relay nucleus.

Johnston (1909) was of the opinion that the nucleus locus coeruleus supplied sensory fibers to the mesencephalic root of the trigeminal nerve, however, May and Horsley (1910) failed to observe chromatolysis in the cells of this nucleus after trigeminal nerve section in the cat and in the monkey. Willems (1911) described the morphology of the nucleus locus coeruleus in detail, distinguishing two parts of the nucleus: one situated medial and dorsal to the superior cerebellar peduncle and which was in synaptic relation with the lateral lemniscus, and one ventromedial to this peduncle and in which terminated numerous longitudinal bundles from the reticular formation. Both portions were traversed by fibers which turn downward from the cerebellum. Arising from the cells of the nucleus locus coeruleus he described fibers which were directed obliquely laterally and rostrally and which pass at right angles through the mesencephalic root of the trigeminal nerve. He did not consider the nucleus locus coeruleus a part of the mesencephalic nucleus of the trigeminal nerve nor did he believe that it gave fibers either to the motor or sensory root of that nerve although he realized that the two nuclei might have some interconnections. Willems proposed that the rostral part of the nucleus which lies dorsolateral to the

decussating brachium conjunctivum was related to acoustic function: "Notons que c'est à ce niveau que a'arrête la dégénérescence consécuitive à la destruction du noyau latéral de l'acoustique."

Kosaka (1912) found no chromatolysis in the nucleus locus coeruleus of the dog following section of the mandibular branch of the trigeminal nerve. He also studied the apparent chromatolysis of this nucleus in the ape following trigeminal nerve section, but concluded that it was the normal appearance of the nucleus rather than true retrograde change. His conclusion was that no part of the trigeminal nerve arose from the cells of the nucleus locus coeruleus.

With the diversity of opinion already reviewed it is pleasant to record the opinion of Merkel (1917) who stated: "Über die Bedeutung der Zellen des Locus caeruleus ist noch nichts Sicheres bekannt." Allen (1919) appears to be the most recent author to persist in deriving a part of the mesencephalic root of the trigeminal nerve from the nucleus locus coeruleus, although Riley (1943) has retained some implication of this in his atlas. Allen thought that the nucleus locus coeruleus was merely the caudal continuation of the mesencephalic nucleus of the trigeminal nerve. Ziehen (1920) described a chief and an accessory nucleus locus coeruleus, although he attributes this subdivision to Meynert. The accessory nucleus corresponds with the nucleus pigmentosus tegmento-cerebellaris of Jacobsohn while the chief nucleus corresponds with the nucleus pigmentosus dissipatus tegmento-pontinus of Jacobsohn. Both of the longer terms seem unnecessary.

Lorente de No' (1922) described fibers of a sensory nature passing from the sensory root of the trigeminal nerve which are seen to enter the nucleus locus coeruleus. He concluded that the nucleus was a part of the trigeminal complex. He cites Bondi as having described a bundle arising from the superior division of the bifurcation of the entering sensory root of the trigeminal nerve which proceeds to the nucleus of polygonal cells in the anterior crus cerebelli. Bondi, according to Lorente de No', considered this an intermediate station between the trigeminal nerve and the cerebellum.

Thelander (1924) described a bundle of fine fibers appearing to arise from the nucleus locus coeruleus in the cat and descending to the level of the inferior olive. These observations were based upon Marchi degeneration studies following destruction

of the nucleus locus coeruleus. It would appear that the tract he described is not Probst's tract and might be a loco-descending tract. Papez (1925a, 1925b) identified a large myelinated bundle of fibers arising from the caudo-lateral aspects of the nucleus locus coeruleus in man. In some instances, he reported, this tract seemed to arise from the caudomedial part of the nucleus instead. In most cases it could be followed as it descended through the metencephalon and myelencephalon and appeared to pass into the spinal cord. In some instances this bundle took a dorsal course and appeared on the floor of the fourth ventricle as a definite white stria associated with the striae medullares. Such a bundle of fibers was first reported by Piccolomini (quoted from Papez, 1925a) and confirmed by numerous other authors. A review of this literature is to be found in a communication by Borghese (1941a, 1941b). The names "conductor sonorus," "cordae tortuosae", "rugae loci caerulei," and "striae of Piccolomini" have been applied to these fibers. Bechterew (1885) considered them as a part of the flocculo-cerebellar connections. Cajal (1909) could not confirm this. Other authors suggested that they formed an inter-connection between the acoustic complex and the trigeminal nuclei and/or the nucleus locus coeruleus. Papez (1925a) gave three principal courses that these fibers might follow. He (Papez, 1925b) proposed that they formed a loco-spinal tract although such a tract had never been encountered in any mammal other than man. Borghese (1941a) denied any direct connection between these fibers and the nucleus locus coeruleus. Barnard and Spann (1946) suggested that they represented a crossed trigemino-cerebellar connection.

Weinberg (1928) described the nucleus locus coeruleus, but could find no contribution of fibers from it to the mesencephalic root of the trigeminal nerve, although the two structures are at certain levels very closely associated. Sheinin (1930) described the nucleus locus coeruleus, but limited it to the region of the aqueduct. Finley and Cobb (1940) discussed the nucleus locus coeruleus and mentioned that Probst had described an efferent tract from this nucleus to the nucleus intercalatus and the dorsal motor nucleus of the vagus. They also mentioned a suggestion made by Hess and Pollak that the nucleus locus coeruleus might subserve some respiratory function, a suggestion made earlier by Lewandowsky (1904).

Riley (1943) in his atlas describes and illustrates five pigmented nuclei in the mesen-metencephalic region of the brainstem: nucleus loci caerulei (nucleus pigmentosus pontis), nucleus accessorius loci caerulei, nucleus nubilis, nucleus pigmentosus (dissipatus) tegmento-cerebellaris, and nucleus pigmentosus tegmento-pontinus. The first three nuclei are the generally recognized nucleus locus coeruleus. The latter two are scattered groups of pigmented neurons lying slightly more caudal than the first pair, but possibly associated with them. Olszewski and Baxter (1954) describe a nucleus locus coeruleus and include the latter two nuclei described by Riley as the nucleus subcoeruleus.

B. Comparative Anatomy. The previous section has dealt almost exclusively with the literature on the nucleus locus coeruleus in man. As early as 1877 Forel had suggested that a homologous nucleus existed in subprimate species, however, the first report of such a nucleus appears to be that of Kljatschkin (1897) who experimented in the cat on a nucleus which he described as the nucleus locus coeruleus. There have been other reports on this nucleus in a wide range of primate and subprimate mammals, but the recent comparative studies of the isthmic and non-tectal midbrain nuclei by Huber and Crosby and their co-workers is the most extensive. In their summary (Crosby and Woodburne, 1943a, 1943e) they homologized the posterior portion of the nucleus lateralis dorsalis tegmenti (dorsolateralis tegmenti) of subprimate species with the nucleus locus coeruleus of primates including man.

Sub-class Marsupialia: Woodburne (1943) described the nucleus lateralis dorsalis tegmenti in the opossum. He reported that the caudal, enlarged portion of this nucleus was divisible into a medial and a lateral portion, both composed of the same medium sized, deeply staining cells. The medial portion was continuous rostrally to the level of the nucleus dorsalis tegmenti and was situated principally in the periventricular gray substance. The lateral portion he homologized with the nucleus locus coeruleus and described it as continuing, mostly in the tegmental gray substance, caudally to the level of the masticator nucleus.

Order Insectivora: Crosby and Woodburne (1943b) identified a nucleus of medium sized, rather deeply staining cells which form an oval mass in the caudolateral region of the nucleus lateralis dorsalis tegmenti of the shrew. This group of cells they described as extending more caudad than does the main mass of

the nucleus and it forms a triangular shaped mass in the angle of the periventricular gray substance. This they considered to be the homolog of the nucleus locus coeruleus. Johnston (1909) identified a nucleus locus coeruleus in the mole, but considered it a part of the mesencephalic nucleus of the trigeminal nerve.

Order Chiorptera: Crosby and Woodburne (1943b) described in the bat an invasion by the caudolateral cells of the nucleus lateralis dorsalis tegmenti into the tegmental gray where these cells are intermingled with those of the underlying reticular nuclei. This region they homologized with the nucleus locus coeruleus.

Order Carnivora: Johnston (1909) and May and Horsley (1910) had described a nucleus locus coeruleus in the cat and Kosaka (1912) identified such a nucleus in the dog. Sheinin (1930) described the cell types of the mesencephalic nucleus of the trigeminal nerve in the dog and was able to differentiate the cells of the nucleus locus coeruleus from those of the mesencephalic nucleus by the arrangement of the Nissl material. He limited the extent of the nucleus locus coeruleus to the region of the cerebral aqueduct, however. Brown (1943a) described the nucleus locus coeruleus in the dog and cat. He reported that in carnivores, as in mammals in general, the nucleus locus coeruleus extends from the oral tip of the dorsal tegmental nucleus to the oral level of the masticator nucleus. Throughout its extent it is located in the angle of the periventricular gray substance and the underlying tegmental reticular formation. The cells, he reported, are often intermingled with those of the mesencephalic nucleus of the trigeminal nerve, however, the slightly smaller size and multipolar shape of the cells of the nucleus locus coeruleus, together with their darker and coarser Nissl granules, serves to differentiate them from the larger, ovoid or round, unipolar cells of the mesencephalic nucleus of the trigeminal nerve. Brown reported that there appeared to be more neurons in the nucleus locus coeruleus in the cat than in the dog, although the nucleus is rather diffuse in both species. Pigment has been reported in older animals of both species (Brown, 1943b).

Tamthai (1943) reported that in the mink the nucleus locus coeruleus appeared as a distinct compact mass of multipolar, medium-sized, triangular or fusiform cells lying ventromedial to the mesencephalic root of the trigeminal nerve. Coarse Nissl granules were scattered throughout the cytoplasm of these cells,

and they had large but irregular nuclei. The cells were without pigment. The nucleus extended from the caudal level of the nucleus profundus mesencephali to the level of the trigeminal complex and was associated with, and often intermingled with, the cells of the mesencephalic nucleus of the trigeminal nerve. He suggested that the nucleus locus coeruleus is the lateral differentiation of the nucleus lateralis dorsalis tegmenti.

Order Rodentia: Gillian (1943a) described a lateral extension of medium sized, deeply staining cells of the nucleus lateralis dorsalis tegmenti in rodents. This region bordered upon the medial surface of the mesencephalic root of the trigeminal nerve and was considered as the homolog of the nucleus locus coeruleus. Kuré (1899) and Willems (1911) described this same region as the nucleus locus coeruleus in the rabbit. Castaldi (1926) reported on this nucleus in the guinea pig and stated that the cells of the nucleus locus coeruleus in that specie were non-pigmented.

Order Artiodactyla: In ungulates Gillian (1943b) described the appearance of a "primative" nucleus locus coeruleus. In the pig the caudal extension of the nucleus lateralis dorsalis tegmenti was closely associated with the mesencephalic nucleus of the trigeminal nerve and it was suggested that this region was the "primative" nucleus. In the sheep this caudolateral portion of the nucleus lateralis dorsalis tegmenti was composed of characteristically medium sized, multipolar, deeply staining cells intermingled with smaller cells of the type found generally in the periventricular gray matter. This nucleus was considered to be the nucleus locus coeruleus, although the cells are non-pigmented. In the horse the same conditions were present although some pigment appeared in some of the cells of the nucleus, particularly in older animals.

Order Xenarthra: Crosby and Woodburne (1943c) described a lateral extension of the nucleus lateralis dorsalis tegmenti in the armadillo. This extension of cells intermingled with the cells of the mesencephalic nucleus of the trigeminal nerve, and they considered it the homolog of the nucleus locus coeruleus. It persists to the level of the entering trigeminal root, and throughout most of its length was situated in the periventricular gray substance and in the underlying tegmental reticular formation.

Order Cetacea: There are no reports available in the literature on this nucleus in the Cetaceans.

Order Primates: In the monkey the cells of the nucleus locus coeruleus are of medium size, fusiform, and have irregular Nissl granules which tend to be located near the periphery of the cell (Finley and Cobb, 1940). The pigmentation and vascularity of the nucleus will be mentioned in the next section. Crosby and Woodburne (1943d) described the cells of the nucleus locus coeruleus in the monkey as of medium size, multipolar, and deeply staining. They were scattered in the periventricular gray and the underlying tegmental reticular formation. The deeper portions of the nucleus they described as poorly separated from the marginal nuclei of the brachium conjunctivum. From the level of the trochlear nerve to the masticator nucleus the nucleus locus coeruleus was situated adjacent to and often intermingled with the cells and fibers of the mesencephalic nucleus and root of the trigeminal nerve. They reported that the nucleus may show some pigmentation in the monkey. May and Horsley (1910) have described the nucleus locus coeruleus in a monkey, and Kosaka (1912) described it in an ape.

Forel (1877) reported on the pigmentation of the nucleus locus coeruleus in man. Cajal's description (1909) of the nucleus in man has already been cited. Crosby and Woodburne (1943d) continued to consider the nucleus locus coeruleus as only the caudal extension of the nucleus lateralis dorsalis tegmenti even in man. They did, however, suggest that the non-pigmented cells found in the part termed nucleus locus coeruleus, and those which lie more rostrally might be included together under a single name. Olszewski and Baxter (1954) in their atlas of the human brainstem have described the nucleus locus coeruleus in some detail. They identify two cell types in this nucleus: "(a) medium sized, round, oval, or multipolar cells with irregular edges, short dendrites, and nuclei which are usually eccentric" and "(b) very small, oval or round, lightly stained cells similar to those which compose the central gray matter."

Sub-mammalian Vertebrates: Few efforts have been made to homologize the nucleus locus coeruleus of mammals with any cell group or groups in other vetebrates. Hoevell (1911) and Kappers (1920) are reported by Huber and Crosby (1936) as having suggested that the nucleus lateralis dorsalis tegmenti (nucleus locus coeruleus) represents an enlarged and dorsolaterally displaced portion or portions of the superior reticular nucleus (lateral reticular nucleus of the pons). Herrick (1914)

described in Amblystoma a neuropil filled region of large cells in the caudal part of the eminentia subcerebellaris tegmenti and interpreted them as a forward continuation of the motor trigeminal nuclei. The position of these cells, however, corresponds closely with the position of the superior reticular nucleus and may represent the region from which the nucleus locus coeruleus may later develop.

C. Pigmentation and Vascularization. Pigmentation, as the name nucleus locus coeruleus implies, is a prominent feature of this nucleus in man and the higher primates. It may occur, however, in other species as well. The earliest description of the pigmentation of the nucleus is that of Reil (1809). The development of the pigmented condition was described by Meynert (1872) who first reported that pigment was absent from the cells of this nucleus in the new-born, but appeared progressively up to the time of puberty. As already mentioned others have referred to the pigmentation and Calligaris (1908) devoted an extensive communication to the subject.

Reports of pigment within the cells of the nucleus locus coeruleus of other mammals have also appeared. Sheinin (1930) observed granules of melanin pigment in the nucleus locus coeruleus of one dog. Gillian (1943b) reported pigment granules in the cells of the nucleus locus coeruleus in a horse. Thelander (1924) found pigment in this nucleus in a cat. Brown (1943b) also observed pigment in the cells of this nucleus in the cat and also in the dog. He described the pigment in both instances as fine granules of melanin. Finley and Cobb (1940) reported that the cells of the nucleus locus coeruleus of the monkey became pigmented at puberty, similar to the condition in man. Adler (1942) found dust-like granules of melanin pigment in all of the cells of the nucleus locus coeruleus of a gorilla.

Another feature of this nucleus is the extreme vascularity first mentioned by Reil (1809). Finley and Cobb (1940) studied the capillary beds of this nucleus in the monkey and concluded that each cell was associated with two capillaries, a situation found elsewhere in the supraoptic and paraventricular nuclei, among larger motor neurons, and among the giganto-pyramidal cells of Betz of the motor cortex. They did not attempt to infer a specific functional significance to this morphological arrangement in the pigmented nucleus locus coeruleus.

III. Methods and Materials

A large number of series of the brainstem of a variety of mammals have been examined in the course of this investigation. Most of these series are the property of the Department of Zoology, Cornell University and I wish to acknowledge their kindness in making this collection available to me. Series of the cat brainstem were prepared and stained for cells with cresyl-echt-violet (Grubler) and for cells and fibers by the methods of Cajal and of Bielschowsky. A series of the brainstem of the sea lion was obtained at Galveston and stained with cresyl-echt-violet (Farbenfabriken Beyer) and by a modified Pal-Weigert method. One of the series of the brainstem of M. rhesus, Foster's M. rhesus No. 1, is the property of Dr. J. W. Papez and I thank him for the permission to study it. Additional macaque series have been made in Galveston and I wish to thank Dr. I. J. Jackson for providing me with these two brains. A number of human cases have been selected from the autopsy service of John Sealy Hospital to provide a series of selected sections of various ages. The latter human and monkey material has been stained with thionine or cresyl-echt-violet (Farbenfabriken Beyer) for cells and by a modified Pal-Weigert or Smith-Quigley method for fibers.

The series of normal material selected for study, description, and illustration include: (A) Homo—A38–5, J. Doe, F. L. (CU)[1]; A–273–53 (KME); BS–Sag., BS–Meyer, 8-Yr. Hor., Foetal–Bodian, and sections from some 20 autopsy cases (UT): (B) Chimpanzee—"Tootsie" and "Fanny" (CU): (C) Baboon—No. 11 (CU): (D) Monkey—Manguebye No. 1 (CU); Forster's M. rhesus No. 1 (JWP); Jackson's M. rhesus No. 1 and No. 2 (UT): (E) Cat—NK, R–16, R–17 (GR); No. 220 (CU): (F) Dog—"Ahem" (CU): (G) Sea Lion—No. 1 (UT): (H) Horse—TS (CU): (I) Sheep—BS and No. 3 (CU): (J) Rabbit No. 1 (CU): (K) Muskrat—No. 1 (CU): (L) Bat—No. 1, No. 13, No. 14 (CU): (M) Opossum—No. 89 (CU): (N) Robin—No. 1 (CU): (O) Owl—No. 1 (JWP).

[1] Abbreviations:

CU—Collection of the Department of Zoology, Cornell University, Ithaca. N.Y.
GR—Collection of the Author.
JWP—Collection of Dr. J. W. Papez.
KME—Collection of Dr. K. M. Earle.
UT—Collection of the Department of Anatomy, The University of Texas Medical Branch, Galveston.

In an effort to establish fiber connections a number of cats were operated upon with the aid of stereotaxic procedures and lesions placed in the region of the nucleus locus coeruleus. After appropriate delay the animals were sacrificed and blocks of the brainstem treated after the method of Schwank and Davenport for degenerating myelin. Two of these cases were suitable and will be described.

IV. Personal Observations

A. Comparative Anatomy. It has been the custom to restrict the use of the term nucleus locus coeruleus to man and those primates in which at least some of the cells of this nucleus, in the adult, contain pigment. The general term nucleus lateralis dorsalis tegmenti (dorsolateralis tegmenti) has been employed in recent years to designate the similarly placed group of cells in those primate and sub-primate mammals in which no pigment is ordinarily found. There is no doubt in the author's mind that these two nuclei are homologous structures, and the continued use of two designations seems superfluous. The individuality of the term nucleus locus coeruleus, despite its literal meaning, and the already large number of named tegmental nuclei has led the author to employ nucleus locus coeruleus in preference to any other term.

In the course of the present investigation the author has studied sections which show the nucleus locus coeruleus in a wide series of mammalian vertebrates. These include: the Virginia opossum, a marsupial; the muskrat and rabbit, both rodents; the small brown bat, a chiroptera; the horse and sheep, both ungulates; a sea lion, a cetacean; the dog, cat, and lion, all carnivores; the Manguebye and macaque monkeys, the chimpanzee, the baboon, and man, all primates. E. C. Crosby and her co-workers have reported on this nucleus in some of these forms and also in the armadillo and in insectivores, the mole and the shrew. These communications have been reviewed. The author has also attempted to identify the homolog of the nucleus locus coeruleus in sub-mammalian vertebrates and has examined several avian, reptilian, amphibian, and fish series. A few such efforts have been made in the past and have been referred to in the previous section.

The review of the literature on the nucleus locus coeruleus, pages 2–11, deals mostly with descriptions of this nucleus in

man. Further comments on the cytoarchitecture of this nucleus in man are in the section on comparative anatomy, pages 15–16. In the human series that I have examined the nucleus locus coeruleus extends from the level of the trochlear nucleus caudally to the rostral level of the masticator nucleus. The cells that form the nucleus are irregularly grouped in the ventrolateral angle of the circumaqueductal and periventricular gray matter and the adjacent tegmental reticular formation (Figs. 1–3).

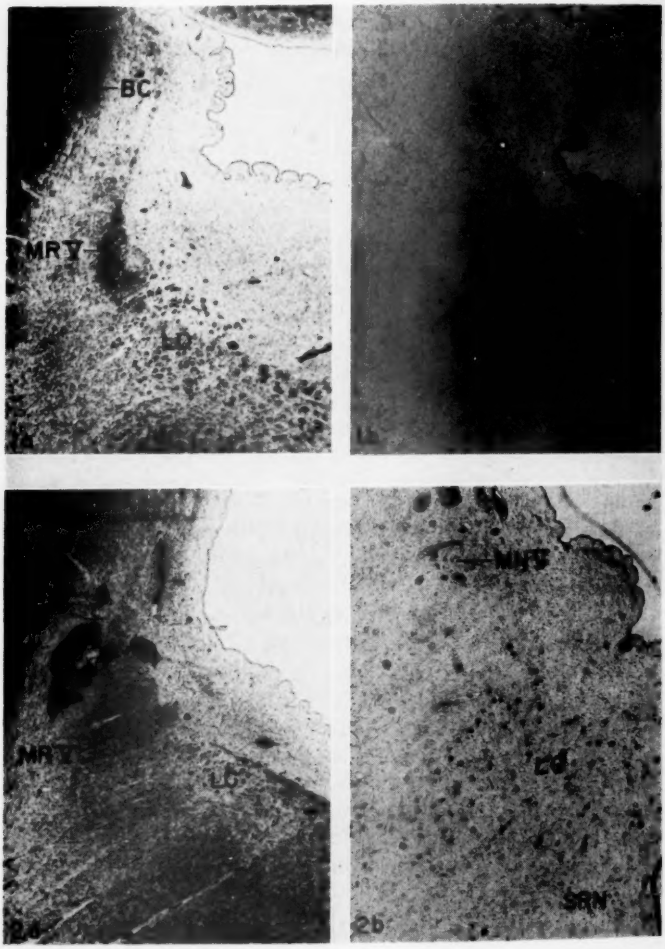
ABBREVIATIONS FOR ALL FIGURES

IV N.—Trochlear nerve.
IV N-X.—Decussation of the trochlear nerve.
BC.—Brachium conjunctivum.
CLL.—Commissure of the lateral lemnicus.
DLF.—Dorsal longitudinal fasciculus.
DTN.—Dorsal tegmental nucleus.
LC.—Nucleus locus coeruleus.
LRS.—Lateral reticulo-spinal tract.
LRT.—Lateral reticulo-thalamic tract.
LTR.—Lateral tegmento-reticular tract.
M V.—Masticator nucleus.
MN V.—Mesencephalic nucleus of the Vth nerve.
MR V.—Mesencephalic root of the Vth nerve.
MRS.—Medial reticulo-spinal tract.
N IV.—Trochlear nucleus.
N VII.—Facial nucleus.
NFO.—Nucleus ovalis.
NIP.—Nucleus isthmi post.
PS V.—Superior sensory nucleus of Vth nerve.
PT.—Probst's tract.
RS.—Rubro-spinal tract.
SRN.—Superior reticular nucleus.
TL.—Afferent fibers from trigeminal complex to nucleus locus coeruleus.
TS.—Tecto-spinal tract.
VM.—Vestibulo-mesencephalic tract.

Throughout the extent of the nucleus it lies ventromedial to the mesencephalic root and nucleus of the trigeminal nerve. The cells of the latter nucleus being large globular neurons are readily distinguished from those of the nucleus locus coeruleus although they are often intermingled with each other (Fig. 4f). Rostrally in the nucleus the cells are all located near the junction between gray matter and reticular formation. As one passes caudally through the extent of the nucleus its cells tend to invade more and more the pretrigeminal tegmental reticular formation. Although the nucleus extends for some distance in the brainstem the majority of its cells lie in the caudal one-half of the nucleus where they are more compactly arranged and the nucleus has a larger cross-sectional area (Fig. 3a).

The cells which form the nucleus locus coeruleus are of two types. The most frequent are of medium size, multipolar, and may be round, oval, or fusiform (Fig. 4). They frequently show an irregular outline and characteristically have their nucleus in an eccentric position. Nissl material, where it is not obscured by

pigment, is seen to be clumped into fairly large masses and arranged peripherally although some smaller granules may be scattered throughout the cytoplasm. The most characteristic feature of the majority of these cells in the adult is the presence of melanin pigment granules. A few cells appear to be free, or al-



FIGS. 1a and 1b. The nucleus locus coeruleus in man at the level of the isthmus rhombencephali (Homo, PM 9991). X–20.

FIGS. 2a and 2b. The nucleus locus coeruleus in man at the caudal level of the mesencephalic nucleus (Homo, PM 9991). X–20.

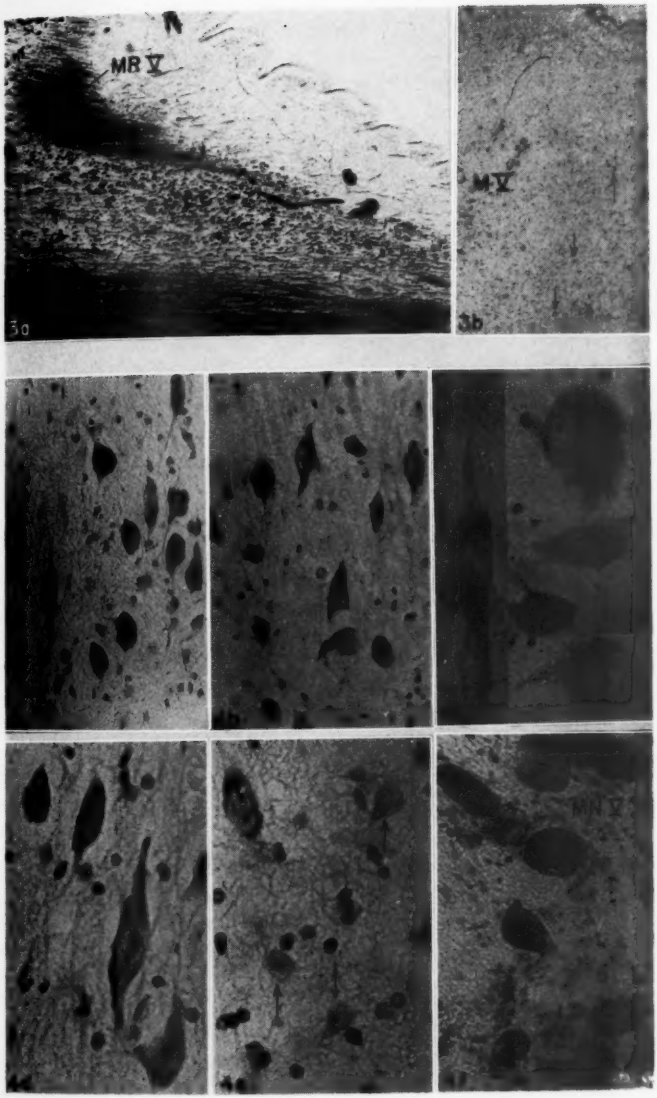


FIG. 3a. Sagittal section showing the nucleus locus coeruleus in man (Homo, PM 10023). X–20.

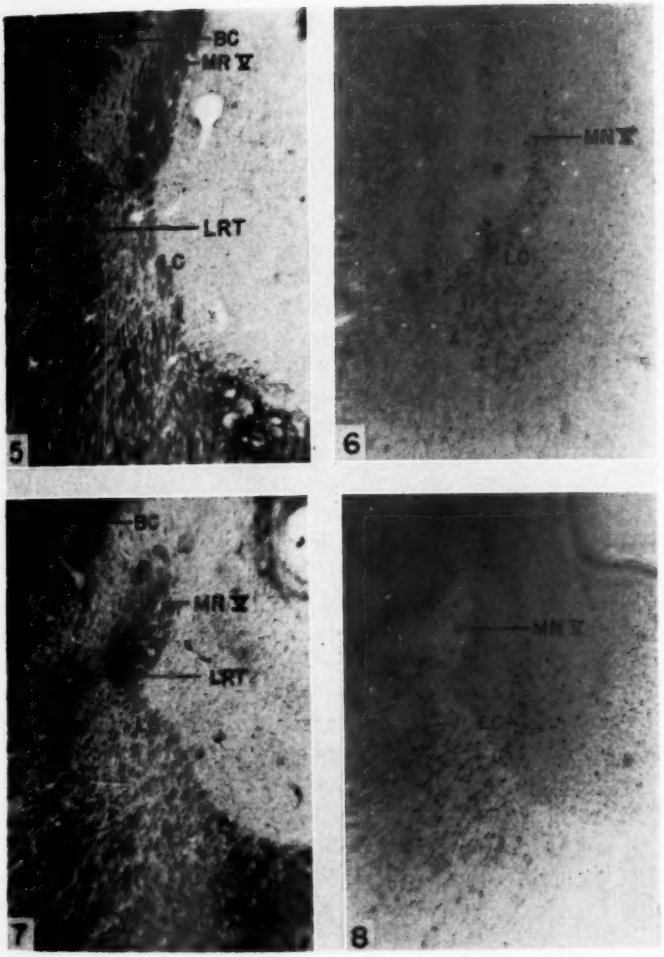
FIG. 3b. Scattered pigmented cells of the nucleus subcoeruleus in man medial and ventromedial to the masticator nucleus (Homo, PM 9991). X–15.

FIG. 4. Cytological detail of the cells of the nucleus locus coeruleus in man: a. Pigmented neurons of nucleus locus coeruleus in the adult, (PM 10045), X–150; b. non-pigmented neurons and early pigmentation in a 9-year-old (PM 10042), X–150; c. pigmented neurons with eccentric position of nucleus and Nissl material clumped at periphery in adult (PM 10045 and A–273–53), X–320; d. non-pigmented neurons with Nissl material clumped at periphery in a 16-month infant (PM 10025), X–320; e. small palely staining neurons of the nucleus locus coeruleus in an adult (A–273–53), X–320; f. intermingling of cells of the nucleus locus coeruleus and of the mesencephalic nucleus of the trigeminal nerve (PM 10023), X–320.

most free, of pigment. Others have small to medium amounts of pigment while many have such a dense accumulation that the cytoplasm is all but obscured (Figs. 4a and 4c). In the human infant brainstems that I have examined (6 days, 2½–3 months, 16 months, and 2 years of age) no pigment has been observed in any of the cells of the nucleus locus coeruleus, and the Nissl material is more frequently distributed throughout the cytoplasm although in many cells peripheral clumping is evident (Fig. 4d). Two cases at 8 and at 9 years of age show that some pigmentation of the cells has occurred (Fig. 4b). The second cell type found in the human nucleus locus coeruleus is a small, oval or round cell with scant, poorly staining cytoplasm (Fig. 4e). These cells are scattered between the larger cells of the nucleus and are generally free of pigment granules. They are very similar to the cell type found in the central gray in which the nucleus locus coeruleus in part lies. However, this small cell type is found in both the reticular and central gray parts of the nucleus.

Caudal to the main mass of the nucleus locus coeruleus a few scattered pigmented neurons are seen medial and ventromedial to the masticator nucleus and spinal nucleus of the trigeminal nerve caudad to the level of the facial nucleus. These form the nucleus subcoeruleus (Olszewski and Baxter, 1954) and are readily identified in most adult human brains (Fig 3b). These cells appear to be the same as the medium sized cells of the nucleus locus coeruleus and lie in the course of the descending lateral tegmento-reticular tract (see pp. 39–40) which originates from the latter nucleus (Johnson and Russell, 1952). It would seem evident that the two nuclei are intimately related.

In the chimpanzee (Figs. 5–8) the nucleus locus coeruleus extends from a level just caudal to the trochlear nucleus caudad to a level about midway in the extent of the masticator nucleus. In the rostral one-third of the nucleus the cells are scattered in the circumaqueductal gray matter and shallowly into the adjacent reticular formation medial and ventromedial to the mesencephalic root of the trigeminal nerve. Here the nucleus is small. In the middle one-third it enlarges rapidly filling the ventrolateral angle of the periventricular gray medial to the mesencephalic root of the trigeminal nerve and invades deeply the underlying tegmental reticular formation. In its caudal one-third the nucleus locus coeruleus rapidly diminishes in size beginning at the rostral level of the masticator nucleus and forms only a small



FIGS. 5 and 6. The nucleus locus coeruleus in the chimpanzee (Chimpanzee "T," Weigert—Sec. 485. Nissl—Sec. 475). X-20.

FIGS. 7 and 8. The nucleus locus coeruleus in the chimpanzee (Chimpanzee "F," Weigert—Sec. 994. Nissl—Sec. 998). X-20.

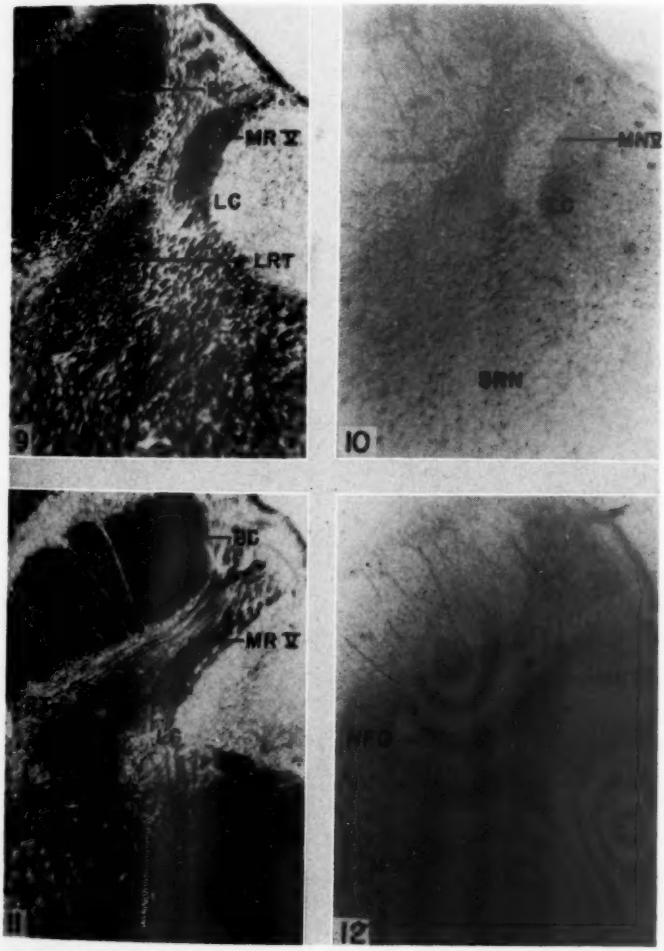
flattened band medial to the mesencephalic root of the trigeminal nerve. The cells are of two types: (1) cells of medium size, multipolar, round or oval, deeply staining, nucleus frequently eccentric, Nissl material finely granular and scattered throughout the cytoplasm with some tendency to clump in larger masses near the periphery of the cell, with a few cells containing melanin pigment granules; and (2) small, round or oval, faintly staining neurons scattered among the cells of medium size.

In the baboon (Figs. 9–10) the nucleus locus coeruleus begins rostrally at the caudal level of the trochlear nucleus. It lies partly in the circumaqueductal gray and partly in the underlying reticular formation. At the level of the decussation of the trochlear nerve it reaches its largest size and invades deeply the tegmental reticular formation ventral to the mesencephalic root of the trigeminal nerve. Caudal to this the nucleus locus coeruleus flattens out against the medial side of the mesencephalic root of the trigeminal nerve, diminishes progressively in size, and terminates caudally about midway in the extent of the masticator nucleus. The cells are very similar to those described in the chimpanzee. They are mostly of medium size, deeply staining, and very compactly arranged. A few small cells are present.

In the Manguebye monkey (Figs. 11–12) the nucleus locus coeruleus extends from the level of the dorsal tegmental nucleus caudally to the caudal level of the masticator nucleus. At its most rostral level it lies ventral to the middle portion of the brachium conjunctivum which obliterates the nucleus as it decussates. As far caudad as the decussation of the trochlear nerve it lies predominantly in the tegmental reticular formation ventral to the mesencephalic root of the trigeminal nerve and to the brachium conjunctivum. As the level of the isthmus rhombencephali is reached the nucleus locus coeruleus gives the appearance of being broken up into several small islets of cells. More caudally in the pretrigeminal region and in the rostral levels of the masticator nucleus it forms a dense mass of cells medial, ventromedial, and ventral to the mesencephalic root of the trigemenal nerve. It rapidly diminishes in size and disappears just caudal to the masticator nucleus. The principal cell type of the nucleus locus coeruleus is like that of the chimpanzee and baboon: of medium size, deeply staining, with eccentric nuclei, and with a few of the cells containing melanin pigment granules. The cells are

very compactly arranged and a few small cells are scattered through the nucleus.

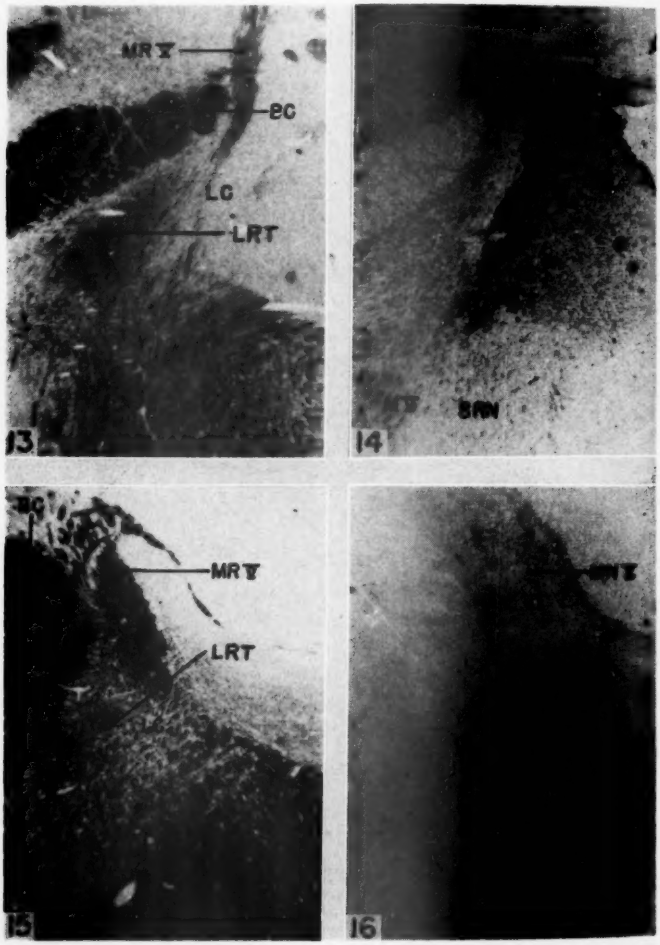
In the macaque monkey (Figs. 13–14) the nucleus locus coeruleus appears rostrally at the level of the dorsal tegmental nucleus where it lies in the ventrolateral angle of the circumaqueductal



FIGS. 9 and 10. The nucleus locus coeruleus in the baboon (Baboon No. 11, Weigert—Sec. 500. Nissl—Sec. 499). X–20.

FIGS. 11 and 12. The nucleus locus coeruleus in the Manguebye monkey (Manguebye No. 1, Weigert—Sec. 361. Nissl—Sec. 363). X–20.

gray ventromedial to the middle and medial portions of the brachium conjunctivum. The nucleus increases rapidly in size and compactness and at the level of the decussation of the trochlear nerve forms a heavy band in the lateral edge of the periventricular gray medial to the mesencephalic root of the trigemi-



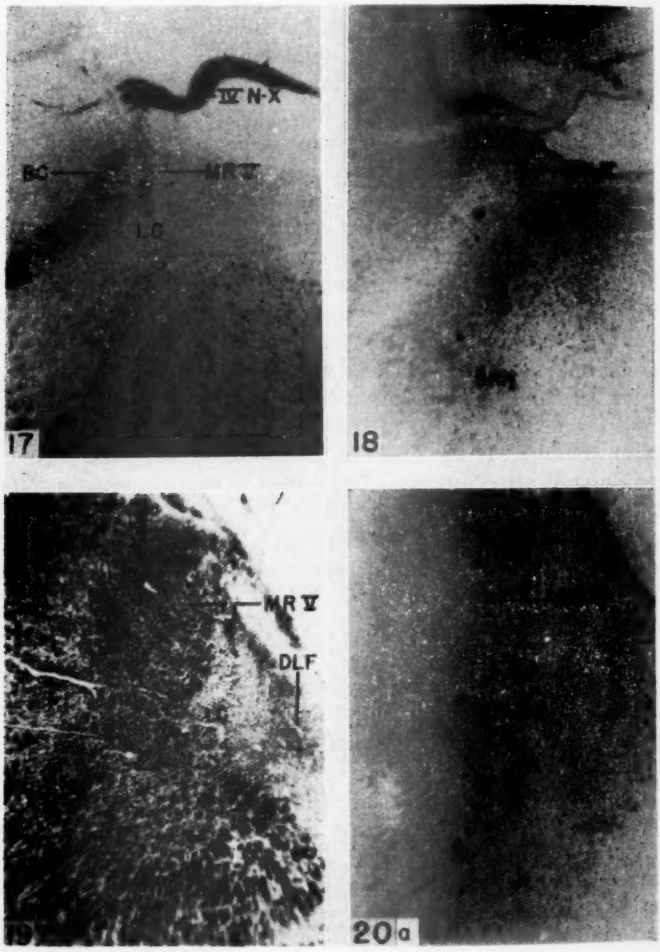
FIGS. 13 and 14. The nucleus locus coeruleus in the macaque monkey (Forster's M. rhesus No. 1, Weigert—Sec. 1130. Nissl—Sec. 1129). X-20.
FIGS. 15 and 16. The nucleus locus coeruleus in the dog (Dog "Ahem," Weigert—Sec. 604. Nissl—Sec. 602). X-20.

nal nerve. It invades the adjacent tegmental reticular formation for only a short distance. The nucleus locus coeruleus then diminishes rapidly in size and terminates caudally at the level of the masticator nucleus. The cells are uniformly of medium size, only a very occasional small cell being seen. The Nissl substance is scattered throughout the cytoplasm although a tendency to clump at the periphery of the cell is noted. The nuclei are frequently eccentric and the cells stain very deeply. An occasional pigmented neuron is seen in the nucleus.

In the dog (Figs. 15–16) and in the cat (Figs. 17–18) the nucleus locus coeruleus extends from the level of the dorsal tegmental nucleus caudad to the middle of the masticator nucleus. Rostrally it lies medial and ventromedial to the brachium conjunctivum in the ventrolateral angle of the circumaqueductal gray and in the underlying tegmental reticular formation. More caudally it lies medial to the mesencephalic root of the trigeminal nerve and invades deeply the tegmental reticular formation rostral to the superior sensory and masticator nuclei of the trigeminal nerve. In both the cat and the dog the cells are of two types. About one-half are of medium size, polygonal or fusiform, with eccentric nuclei, and containing coarse deeply staining Nissl granules. The remainder are of small size with sparse, poorly staining cytoplasm. The entire nucleus is less compact than in the primates, but is still readily defined. In a few sections of the brainstem of the lion (Felis leo) that were available it seems evident that the nucleus locus coeruleus has a similar extent and cytoarchitecture to that seen in the other two carnivores just described.

In the sea lion (Figs. 20b–20c) the nucleus locus coeruleus begins rostrally at the rostral levels of the trochlear nucleus where it appears to be separated into two segments, one in the central gray matter medial to the medial part of the brachium conjunctivum and the second within the tegmental reticular formation ventral to the medial part of the brachium conjunctivum. Passing caudally the two segments fuse to form a comma-shaped mass with the punctate part of the nucleus lying in the circumaqueductal gray between the dorsal tegmental nucleus and the medial edge of the brachium conjunctivum, and the tail swinging into the tegmental reticular formation ventral to the brachium conjunctivum. At the level of the exciting trochlear nerve the portion of the nucleus locus coeruleus lying within the peri-

ventricular gray has become somewhat smaller and lies medial to the mesencephalic root of the trigeminal nerve. The more lateral portion has increased rapidly in size and lies in the tegmental reticular formation ventral to the median one-half of the brachium conjunctivum. This appearance persists to the rostral

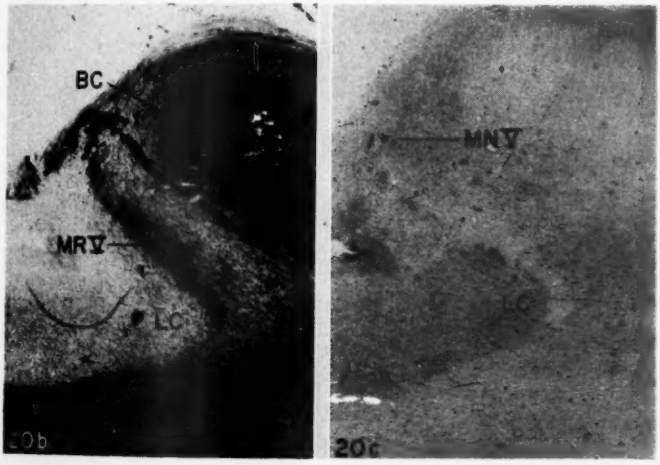


FIGS. 17 and 18. The nucleus locus coeruleus in the cat (Cat NK, Weigert—Sec. 616. Nissl—Sec. 615). X–20.

FIGS. 19 and 20a. The nucleus locus coeruleus in the horse (Horse "TS," Weigert—Sec. 1441. Nissl—Sec. 1440). X–20.

levels of the masticator nucleus. From this level caudally the nucleus diminishes progressively in size, the lateral portion disappearing first so that the most caudal portions of the nucleus locus coeruleus occupy a small wedge in the ventrolateral angle of the periventricular gray just caudal to the mesencephalic root of the trigeminal nerve. The cells are of two types, the most prominent being of medium size; round, oval, or fusiform; and with coarse, rather deeply staining Nissl granules which may be scattered throughout the cytoplasm or tend to clump near the periphery of the cell. There are many smaller cells scattered throughout the nucleus. The nuclei of most of the cells tend to be centrally located, although an eccentric location in some of the medium sized cells is not uncommon.

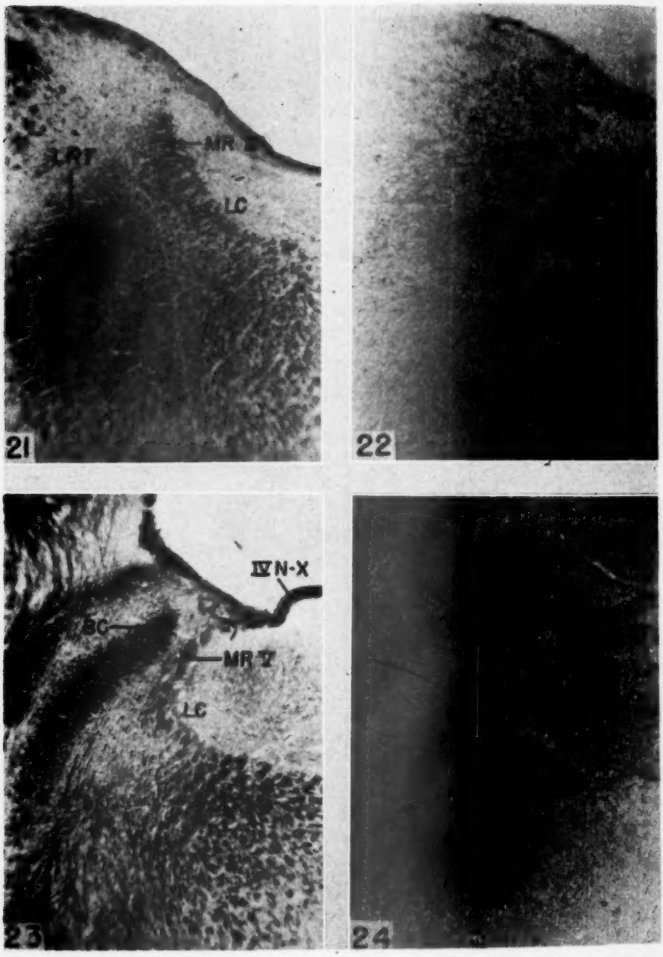
In the horse (Figs. 19–20a) the nucleus locus coeruleus appears rostrally in the lateral wall of the periventricular gray just



FIGS. 20b and 20c. The nucleus locus coeruleus in the sea lion (Sea lion No. 1, Weigert—Sec. 280. Nissl Sec. 281). X-15.

rostral to the dorsal tegmental nucleus. Further caudad it surrounds the ventral part of the mesencephalic root of the trigeminal nerve and extends into the tegmental reticular formation. It reaches caudally to the level of the entering motor root of the trigeminal nerve. The cells of the nucleus are mostly of small size with a few medium sized, deeply staining neurons scattered

throughout the nucleus. A few of the medium sized cells contain some granules of melanin pigment. The nucleus is poorly defined in the horse and the cells not as compact as in the carnivores. In the sheep (Figs. 21–22) the nucleus lies in the same position as in the horse. Again the cells of smaller size predomi-



FIGS. 21 and 22. The nucleus locus coeruleus in the sheep (Sheep "BS," Weigert—Sec. 885. Sheep No. 3, Nissl—Sec. 216). X–20.
FIGS. 23 and 24. The nucleus locus coeruleus in the rabbit (Rabbit No. 1, Weigert—Sec. 229. Nissl—Sec. 230). X–20.

nate with only a few scattered medium-sized cells. The nucleus locus coeruleus of the sheep is the least well defined of those that I have observed.

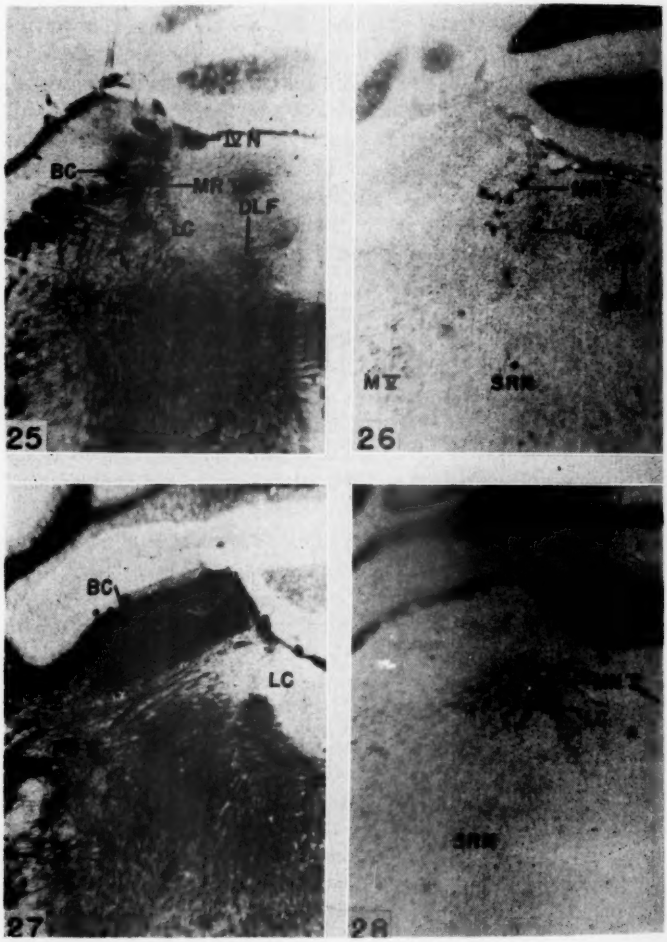
In the rabbit (Figs. 23–24) the nucleus locus coeruleus extends from the level of the dorsal tegmental nucleus caudally to a point midway in the extent of the masticator nucleus. The nucleus is larger in its rostral one-half, the caudal portion forming only a small triangular mass medial to the mesencephalic root of the trigeminal nerve. The cells are compactly arranged, particularly in the more rostral sections, and are of both small and medium size. In the muskrat (Figs. 25–28) is a small, but well defined nucleus as in the rabbit. It extends from the rostral level of the dorsal tegmental nucleus to the level of the masticator nucleus lying almost entirely in the periventricular gray. The cells are of small and of medium size, stain deeply, and are compactly arranged.

In the bat (Figs. 29–31) the nucleus locus coeruleus is an ovoid mass of medium sized cells lying in the angle of the periventricular gray. The nucleus is small extending for only a short distance rostral and a short distance caudal to the dorsal tegmental nucleus. It lies ventral and ventromedial to the mesencephalic root of the trigeminal nerve with most of its cells lying in the periventricular gray, although a few invade the adjacent tegmental reticular formation.

In the opossum (Fig. 32) the nucleus locus coeruleus extends from the level of the dorsal tegmental nucleus caudad to the level of the entering trigeminal root. At almost all levels it forms an oval mass surrounding the ventral part of the mesencephalic root of the trigeminal nerve. In this species the commissure of the lateral lemniscus passes through the rostral levels of the nucleus and appears to be closely associated with the rich neuropil of the nucleus locus coeruleus (Fig. 44).

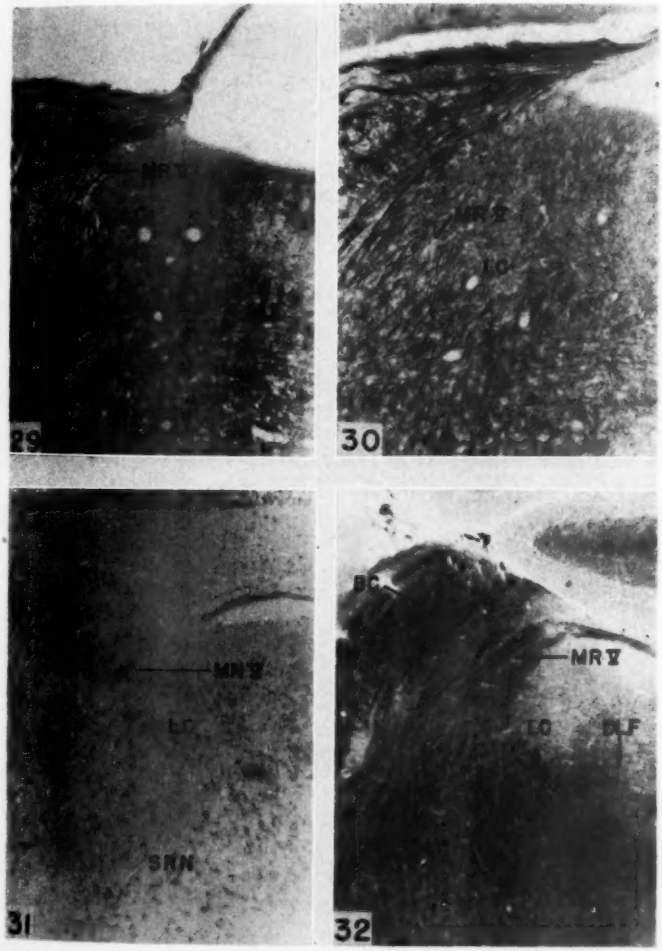
In submammalian vertebrates there have been only a few efforts to identify or to homologize any nuclear mass with the nucleus locus coeruleus of the mammalian brainstem. I have examined three series of the brainstem of birds, two of the owl, and one of the robin. In the former (Figs. 33–40) there appears a group of cells which lie just on and beneath the floor of the periventricular gray in the trigeminal and pretrigeminal tegmentum. Rostrally this nucleus first appears in the tegmental reticular formation (Figs. 33–34) at the level of the nucleus isthmi

posterior (nucleus of the isthmio-optic tract). More caudad the cells of this nucleus invade dorsally into the periventricular gray as the nucleus increases in size. At the rostral levels of the masticator nucleus it occupies a large part of the periventricular gray and invades deeply into the adjacent tegmentum (Figs. 35–38).



FIGS. 25 and 26. Rostral end of the nucleus locus coeruleus in the muskrat (Muskrat No. 1, Weigert—Sec. 228. Nissl—Sec. 227). X–20.
FIGS. 27 and 28. Caudal end of the nucleus locus coeruleus in the muskrat (Muskrat No. 1, Weigert—Sec. 197. Nissl—Sec. 198). X–20.

At the caudal level of the mesencephalic root this nucleus forms a triangular mass in the periventricular gray and adjacent tegmentum medial to this root. The nucleus disappears near the caudal level of the masticator nucleus. Its cells are of medium



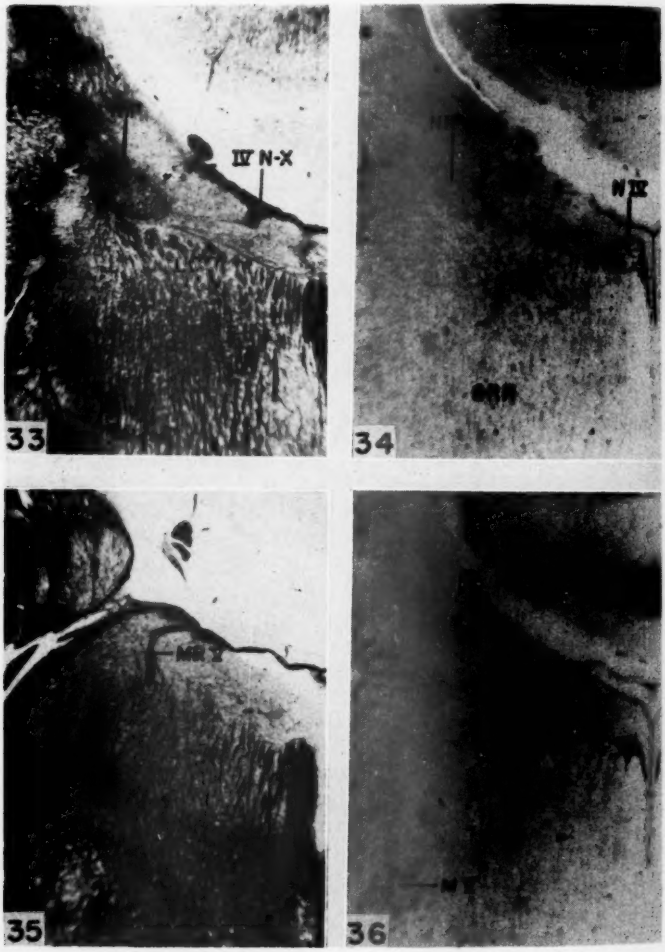
FIGS. 29 and 30. The nucleus locus coeruleus in the bat (Bats 14–5 and 1–9. Cajal). X–30.

FIG. 31. The nucleus locus coeruleus in the bat (Bat 13–12. Nissl). X–30.

FIG. 32. The nucleus locus coeruleus in the opossum (Opossum No. 89, Weigert—Sec. 291). X–20.

and small size and stain very deeply. A similar, although less well defined, nucleus is seen in the robin.

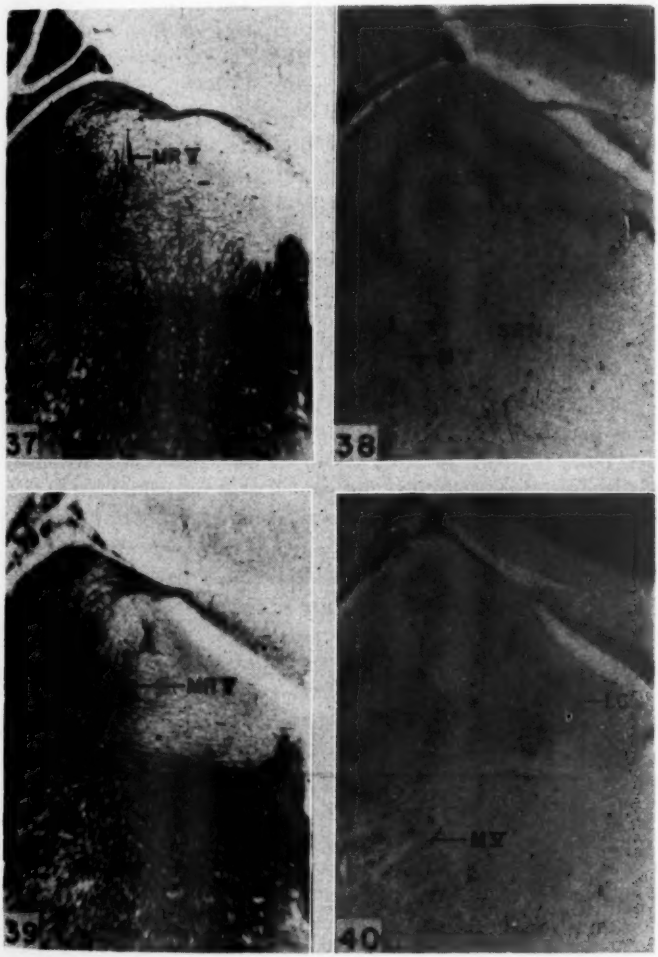
This nucleus in the birds appears to correspond to two nuclei found in mammals: the nucleus reticularis superior (lateral reticular nucleus of the pons, nucleus pontis centralis oralis) and



FIGS. 33 and 34. The nucleus locus coeruleus in the owl at the level of the nucleus isthmi posterior (Owl No. 1. Weigert—Sec. 500. Nissl—Sec. 499). X–20.

FIGS. 35 and 36. The nucleus locus coeruleus in the owl at the rostral level of the masticator nucleus (Owl No. 1. Weigert—Sec. 512. Nissl—Sec. 511). X–20.

the nucleus locus coeruleus. It has already been suggested that the nucleus locus coeruleus is derived from the superior reticular nucleus (Kappers, 1920, and Hoevell, 1911). The observations reported here in the owl and robin would strongly support this



FIGS. 37 and 38. The nucleus locus coeruleus in the owl at the rostral level of the superior sensory nucleus of the trigeminal nerve (Owl No. 1. Weigert—Sec. 517. Nissl—Sec. 516). X–20.

FIGS. 39 and 40. The nucleus locus coeruleus in the owl at the caudal level of the mesencephalic root of the trigeminal nerve (Owl No. 1. Weigert—Sec. 522. Nissl—Sec. 523). X–20.

view. Further support is gained from the following observations of apparent fiber connections which may be seen in the myelin-stained figures: (1) afferent fibers from the superior sensory nucleus of the trigeminal nerve and perhaps from the nerve itself (Figs. 37 and 39); (2) neuropil in the dorsal part of the nucleus closely related to the dorsal longitudinal fasciculus; and (3) apparent coursing of efferent fibers from the nucleus ventrally into the tegmental reticular formation (Fig. 37). The presence of these connections to and from the mammalian nucleus locus coeruleus will be discussed in the following section.

B. Connections. In the review of the literature the numerous reported connections of the nucleus locus coeruleus have been mentioned. Various authors have reported afferent fibers to the nucleus from the ipsilateral trigeminal nerve or from its superior sensory nucleus. The best description of these fibers is that of Lorente de No' (1922). Papez and Rundles indicated them in a figure (Papez and Rundles, 1937, Fig. 1). Such fibers are readily seen and may be identified in figures 11, 40–43. Poinatowsky (1892) suggested a crossed bundle of fibers to the nucleus locus coeruleus from the contralateral trigeminal complex, however, I have no evidence for such a connection although the wealth of interconnections between the superior sensory and masticator nuclei of the two sides would permit such a situation.

Cajal (1909) stated that the nucleus locus coeruleus lies in a "very extensive diffuse interstitial plexus" (neuropil) which extends throughout the nucleus and is continuous with a similar plexus within the mesencephalic nucleus of the trigeminal nerve. He believed that this plexus was continuous from the region of the vagal nuclei to the region of the nucleus locus coeruleus just in and beneath the floor of the periventricular gray. I have observed this neuropil much as Cajal described it in all the myelin-stained and silver preparations that I have studied.

The origin of a part of the dorsal longitudinal fasciculus from the hypothalamus is generally accepted. Crosby and Woodburne (1951) have made an extensive study of this tract in the monkey. I have repeatedly observed that the cells of the nucleus locus coeruleus lying in the central gray are in close contact with the lateral part of this tract, a relationship well seen in figures 7, 11, 15, 19, 23, 25, and 32. This observation confirms the observations of Crosby and Woodburne that among the components of the dorsal longitudinal fasciculus recognizable were "...(d) fascicles

arising and (or) terminating in . . . the laterodorsal tegmental nucleus. . . ."

The origin and course of the archistriato-mesencephalic tract was described by Edinger (1895) and has since been termed variously occipito-mesencephalic tract (Edinger, 1895; Edinger, *et al.*, 1903; Schroder, 1911; and Huber and Crosby, 1929), ventral strio-mesencephalic tract (Gröbbels, 1924), and archistriato-mesencephalic tract (Herman, 1925; and Craige, 1930). This tract, only described as high as the birds, arises in the amygdalar region, passes into the diencephalon via the inferior thalamic peduncle, and then descends to the tegmental reticular formation in the region of the trigeminal nuclei. In its terminal portion it courses in the region in which the sub-mammalian homolog of the nucleus locus coeruleus is presumed to lie. The neurophysiological reports of Kabat (1936) and others strongly suggest that a homolog of this tract exists in mammals, and, if so, would be presumed to have some terminal connections with the nucleus locus coeruleus.

Herrick (1914) described a nucleus in Amblystoma which he thought to be the homolog of the nucleus locus coeruleus in mammals. He described a tractus visceralis ascendens ending in the vicinity of this nucleus. He further reported that the prefacial portion of the fasciculus solitarius ends just medial to this nucleus and although he reported no evidence of direct connections between these two structures their proximity to the presumptive region of the homolog of the nucleus locus coeruleus was suggestive. The prefacial portions of the nucleus and fasciculus solitarius, the nucleus and fasciculus ovalis (of Ziehen), have been described in detail by Nagoette (1906) and are described by Olszewski and Baxter (1954). In some instances it would appear that these structures reach the caudal levels of the nucleus locus coeruleus where they lie just ventrolateral to that nucleus (Figs. 11–12, 43) and some connection between the two would not be impossible. These would represent an afferent visceral contribution from the facial nerve to this nucleus.

In some sections through the level of the commissure of the lateral lemniscus it would appear that this tract gives fibers to the nucleus locus coeruleus. Such a relationship is especially well seen in the opossum (Fig. 44) and would confirm a connection previously proposed by Willems (1911).

The efferent connections of the nucleus locus coeruleus have been considered by many authors. This has led to many conflicting ideas and theories. In order to present some experimental data bearing upon this problem a number of cats were operated

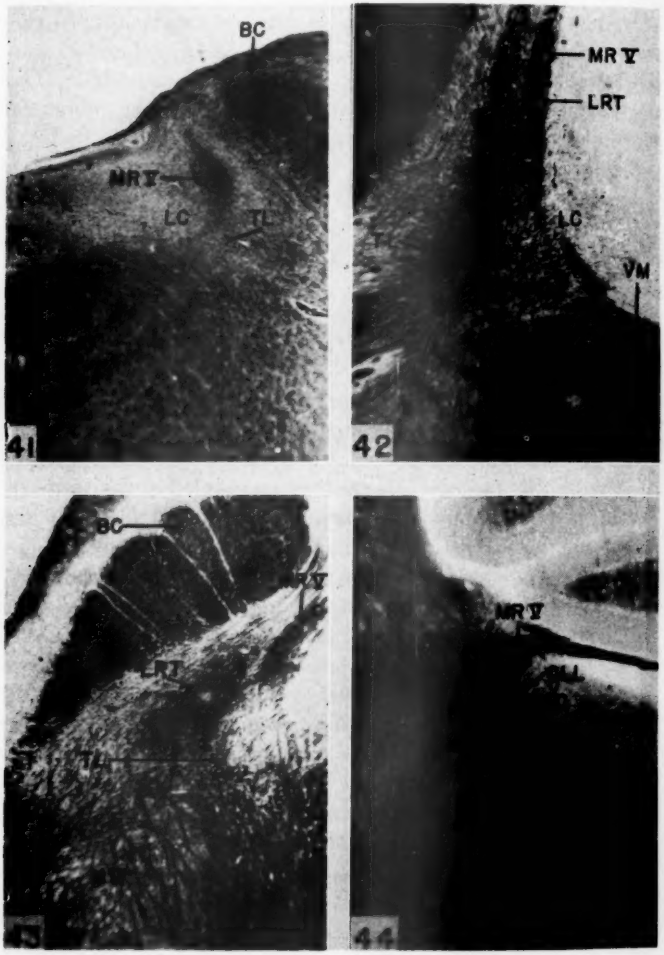


FIG. 41. Trigemino-locus fibers in the cat (Cat 220, Sec. 384). X–20.
FIG. 42. Trigemino-locus fibers in man (Homo A38–5, D7). X–20.
FIG. 43. Trigemino-locus fibers in the monkey (Manguebye No. 1, Sec. 361). X–20.
FIG. 44. Relationship between the commissure of the lateral lemniscus and nucleus locus coeruleus in the opossum (Opossum No. 89, Sec. 304). X–20.

upon in an effort to destroy the nucleus locus coeruleus. In two animals the lesions were excellent and they have been selected for description. Other of the animals provided both positive and negative evidence to support the interpretation derived from these two animals.

Cat R-16 (Figs. 45–48). Lesion: Single electrolytic lesion placed by stereotaxic methods in left side of brainstem and after 14 days post-operative survival a Marchi series of the brainstem

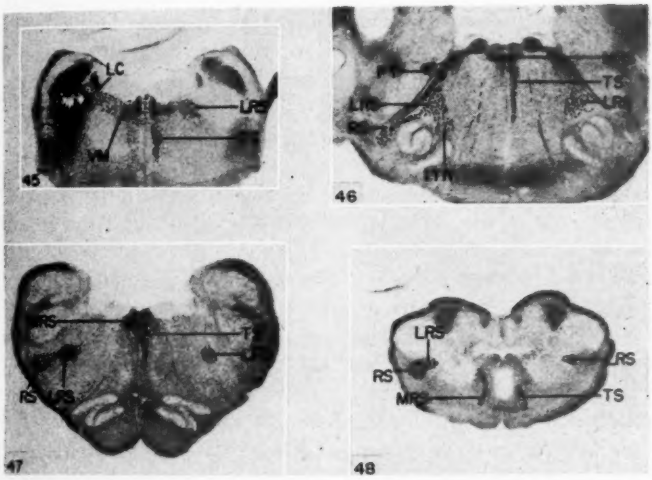


FIG. 45. Lesion in cat R–16 showing nucleus locus coeruleus destroyed on the left side (Cat R–16, Sec. 390. Marchi).
FIG. 46. Lateral tegmento-reticular tract at the level of the facial nerve (Cat R–16, Sec. 530. Marchi).
FIG. 47. Lateral tegmento-reticular tract at the level of the inferior olivary complex (Cat R–16, Sec. 780. Marchi).
FIG. 48. Degenerating tracts below the level of termination of the lateral tegmento-reticular tract (Cat R–16, Sec. 980. Marchi).

prepared. Lesion began at level of commissure of the lateral lemniscus involving at this level the middle one-half of the brachium conjunctivum, the lateral reticulo-thalamic tract, and the tegmental reticular formation immediately adjacent to these structures. The needle track at this level and higher levels has interrupted the commissure of the lateral lemniscus and the tecto-spinal tract. At the rostral level of the masticator nucleus the lesion has enlarged to include the ventral two-thirds of the brachium conjunctivum and the tegmental portion of the nucleus locus coeruleus. Slightly further caudad only a small part of the

ventro-medial portion of the brachium conjunctivum is involved. Slight involvement of the rostral end of the superior sensory nucleus of the trigeminal nerve, the dorsal portion of the masticator nucleus, and the ventral portion of the mesencephalic root and nucleus of the trigeminal nerve, together with almost complete destruction of the nucleus locus coeruleus was effected. Caudal to this the lesion rapidly diminishes in size and ends at the level of the emerging mesencephalic root of the trigeminal nerve.

Degeneration: Rostral to the lesion degeneration is seen in the homolateral lateral reticulo-thalamic tract, in the homolateral vestibulo-mesencephalic tract, and in the brachium conjunctivum. At the rostral level of the lesion degeneration is seen in the homolateral median longitudinal fasciculus and lateral lemniscus, in the ventral decussation and descending limb of the brachium conjunctivum, in the commissure of the lateral lemniscus, and in the contralateral tectospinal tract. At the rostral level of the masticator nucleus (Fig. 45) three additional tracts or systems are degenerated: (1) homolateral fibers passing dorsad medial to the brachium conjunctivum and entering the anterior medullary velum; (2) degenerating fibers emerging from the anterior medullary velum and passing into the contralateral pretrigeminal region, the head of the superior sensory nucleus of the trigeminal nerve, and into the lateral reticular formation ventromedial to the latter nucleus; (3) degeneration of fibers emerging from the dorsomedial aspect of the lesion and decussating through and under the median longitudinal fasciculus and coursing into the contralateral dorsolateral tegmentum. Levels through the trigeminal nuclei show the latter degeneration to be prominent, and it is presumed to include commissural fibers between the trigeminal nuclei of the two sides, the decussation of the lateral reticulo-spinal tract described by Papez (1926) in the cat, and a suggestion of fibers passing into the contralateral nucleus locus coeruleus. Caudal to the lesion (Figs. 46–48) the following tracts are seen to be degenerated and may be traced: (1) the homolateral Probst's tract descending dorsolateral to the exiting facial nerve and then ventromedial to the fasciculus solitarius as far as the caudal levels of the facial nucleus; (2) the homolateral rubro-spinal tract interrupted by the lesion descending into the spinal cord; (3) a small portion of the homolateral medial reticulo-spinal tract; (4) the contralateral lateral reticulo-spinal

tract; (5) the homolateral lateral reticulo-spinal tract interrupted in passage; and (6) previously unidentified fibers which first accompany the latter tract, then lie ventromedial to it, and appear to terminate in the ventral portion of the reticular formation dorsal to the inferior olivary complex on the same side as the lesion. The last tract is identified as the lateral tegmento-reticular tract (Johnson and Russell, 1952).

Cat R-17 (Figs. 49–56). Lesion: Single electrolytic lesion placed by stereotaxic methods in the right side of the brainstem. After 14 days post-operative survival a Marchi series of the

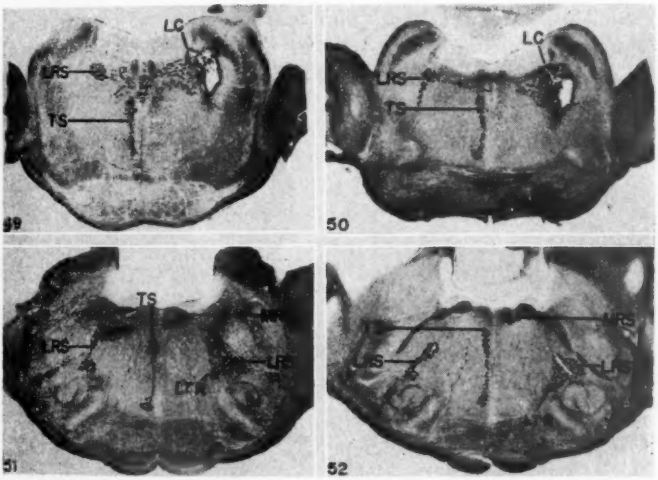


Fig. 49. Lesion in cat R–17 showing nucleus locus coeruleus destroyed on the right side (Cat R–17, Sec. 340. Marchi).
Fig. 50. Lesion in cat R–17 showing nucleus locus coeruleus destroyed on the right side (Cat R–17, Sec. 370. Marchi).
Fig. 51. Lateral tegmento-reticular tract dorsal to the superior olivary complex (Cat R–17, Sec. 440. Marchi).
Fig. 52. Lateral tegmento-reticular tract at the level of the facial nerve (Cat R–17, Sec. 480. Marchi).

brainstem was prepared. Lesion begins at rostral level of the decussation of the trochlear nerve involving at this level the middle one-half of the brachium conjunctivum, commissure of the lateral lemniscus, lateral reticulo-thalamic tract, and adjacent reticular formation. The needle track interrupted tecto-spinal fibers at a higher level. Caudally (Fig. 49) the lesion expands to destroy the middle one-fourth of the brachium conjunctivum, the entire nucleus locus coeruleus, and the lateral part of the superior

reticular nucleus. At this level the lesion involves the region of the reticular formation through which the lateral reticulo-spinal tract from the contralateral superior reticular nucleus courses.

Degeneration: Rostral to the lesion degeneration is seen in the fibers of the homolateral lateral reticulo-thalamic tract, tecto-spinal and tecto-pontine tracts, and in the brachium conjunctivum. In sections at the rostral end of the lesion degenerated fibers are seen in the decussation of the descending limb of the brachium conjunctivum, in the tecto-spinal tract, and in the commissure of the lateral lemniscus. Further caudal (Figs. 49–50) degeneration is seen in fibers crossing the midline from the medial aspect of the lesion and coursing into the contralateral tegmental reticular formation in the position of the lateral reticulo-spinal tract. Caudal to the lesion (Figs. 51–56) degener-

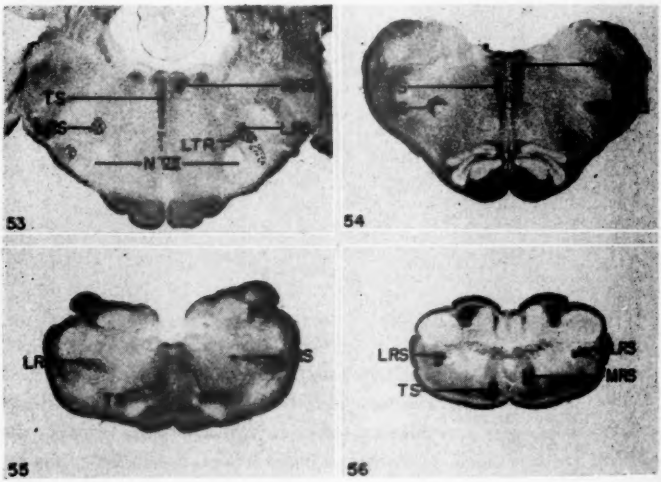


FIG. 53. Lateral tegmento-reticular tract at the level of the facial nucleus (Cat R–17, Sec. 540. Marchi).

FIGS. 54–56. Degeneration of fiber tracts below the level of termination of the lateral tegmento-reticular tract in the medullary reticular formation dorsal to the inferior olivary complex (Cat R–17, Secs. 640, 800, and 920. Marchi).

ation is seen in the following tracts and may be traced: (1) in both lateral reticulo-spinal tracts, (2) in the homolateral medial reticulo-spinal tract, (3) perhaps some fine descending degeneration in the homolateral dorsal longitudinal fasciculus, and (4) in the previously unidentified system of fibers observed in

Cat R-16 and identified as the lateral tegmento-reticular tract. This latter group of fibers appears to emerge from the caudal region of the nucleus locus coeruleus and course ventral and caudad mixed with the fibers of the lateral reticulo-spinal tract. At the level of the facial nucleus they swing medially, occupy a position ventromedial to the latter tract, and appear to terminate in the ventral medullary reticular formation dorsal to the inferior olivary complex. Probst's tract was not degenerated in this animal.

V. Discussion and Conclusions

In this investigation I have attempted an extensive comparative study of the anatomy of the nucleus locus coeruleus in a wide range of mammals together with an attempt to describe the probable homolog of this nucleus in avian forms. Experimentally placed lesions were made in cats and the animals treated by the Marchi method in an effort to establish the fiber connections of this nucleus. As a preface to this study a detailed review of the literature was undertaken with the development of a remarkable diversity of opinion expressed by various authors concerning the connections and the function of the nucleus locus coeruleus since the time that it was first described by the brothers Joseph and Karl Wenzel in 1811. The name substantia ferruginea, introduced by Arnold in 1838, has been employed by a few authors to designate this nucleus in man. Castaldi in 1926 introduced the term nucleus dorsolateralis tegmenti for the homologous nucleus in the subprimate mammals, and this terminology was followed by E. C. Crosby and her co-workers. Here the term nucleus locus coeruleus is employed in all forms described.

The nucleus locus coeruleus is a prominent feature of the dorsolateral tegmental reticular formation in the trigeminal and pre-trigeminal levels of the brainstem of all mammals studied. The nucleus extends from about the level of the dorsal tegmental nucleus or about the caudal level of the trochlear nucleus caudad to about the rostral level of the masticator nucleus. It lies in the ventrolateral angle of the circumaqueductal and periventricular gray matter and invades, often deeply, the underlying tegmental reticular formation of the metencephalon. In its more caudal portions the nucleus locus coeruleus usually appears to lie close to or intermingled with the cells and fibers of the mesencephalic nucleus and root of the trigeminal nerve.

The nucleus locus coeruleus is composed of two cell types in most mammals. In general the predominent cell type is of medium size; round, oval, or fusiform; multipolar; rather deeply staining; and with the nucleus frequently eccentric. In most species there is a tendency for some if not all of the cells to have the Nissl granules clumped in rather coarse granules near the periphery of the cell. The second cell type is a small neuron which is oval, multipolar, and palely staining and is of the type generally found in the central gray matter of the brainstem. These small cells are usually fewer in number than the medium sized cells and are scattered throughout the nucleus.

In the medium sized cell type of adult primates, particularly in man, the cytoplasm is filled with finely divided granules of melanin pigment. This pigmented condition has appeared by puberty although no evidence of pigmentation has been observed during the first several years of life in man. Infrequently, in older individuals of sub-primate species some pigment granules are seen in the cells of the nucleus locus coeruleus.

The close morphological relationship between the nucleus locus coeruleus and the mesencephalic root and nucleus of the trigeminal nerve has in the past led to widely divergent opinions on the connections and functions of the nucleus locus coeruleus. Thus Meynert (1872), Obersteiner (1893), Kölliker (1896), and as recently as Allen (1919) and Riley (1943) reported that fibers pass from the nucleus locus coeruleus into the mesencephalic root of the trigeminal nerve. On the other hand, Kohnstamm (1910), May and Horsley (1910), Kosaka (1912), and other more recent authors have denied such a connection. I have been unable to identify any such connection in any of the experimental animals studied during the course of this investigation. I concur with Sheinin (1930) that the differences of opinion on this point may probably be traced to inconsistencies in delimiting the nucleus locus coeruleus. With the exception of afferent fibers to the nucleus locus coeruleus from the trigeminal nerve and its sensory nuclei, it must be presumed that the nucleus locus coeruleus is not a part of the central somesthetic mechanism for the head and is not a part of the trigeminal complex.

Other afferent connections to the nucleus locus coeruleus have been discussed (see pp. 30 to 32). Interconnections between the two nuclei have been suggested, but the material that I have examined fails to establish clearly any such connection. The af-

ferent connections of the nucleus locus coeruleus may be summarized as: (1) fibers from the trigeminal nerve and from the superior sensory and mesencephalic nuclei of that nerve; (2) a diffuse interstitial plexus that lies in the ventrolateral angle of the periventricular gray and the immediately adjacent tegmental reticular formation and which is continuous from the level of the vagal nuclei to the level of the nucleus locus coeruleus, *i.e.*, the extent of the pontine and medullary tegmentum; (3) collaterals from the dorsal longitudinal fasciculus; (4) collaterals from the commissure of the lateral lemniscus; and (5) a suggested homolog of the archistriato-mesencephalic system descending from the amygdaloid complex.

Many efferent connections have been described for the nucleus locus coeruleus. Homen (1890) has described degenerating fibers from this nucleus passing caudally in the median longitudinal fasciculus, but it is probable that he misidentified fibers of the medial reticulo-spinal system originating from the superior reticular nucleus which lies subjacent to the caudal portion of the nucleus locus coeruleus. Papez (1925b) described a definite locospinal tract forming a prominent stria on the floor of the IVth ventricle. This tract he believed to be a part of the system of medullary striae often termed "conductors sonorus" or "striae of Piccolomini." Cajal (1909) and Probst (according to Finley and Cobb, 1940) mentioned some poorly defined, thin fibers passing from the nucleus locus coeruleus caudally in the periventricular gray. These latter two tracts may be different forms of the same system and may account for the fine degeneration observed in the dorsal longitudinal fasciculus in Cat R-17. The only previous report of a definite descending tract which would appear to correlate with the previously unnamed lateral tegmento-reticular tract observed in Cats R-16 and R-17 is found in a communication of Thelander (1924). He described a fine descending fiber system from the nucleus locus coeruleus in the cat. It coursed in the lateral reticular formation to the level of the inferior olivary complex, but the author failed to designate it as a particular tract.

When the experimental material here described was first studied the difference in the appearance and position of the lateral reticulo-spinal tracts on the two sides was not fully appreciated. I am deeply indebted to Dr. Frederic H. Johnson, at that time my associate, for the careful study and critical judgment

which he brought to bear on this problem and which contributed significantly to the final determination that a second system of fibers was involved. The origin and course of the lateral reticulo-spinal tract in the cat has been well described by Papez (1926). The second system of fibers, named the lateral tegmento-reticular tract by Johnson and Russell (1952), takes origin from the cells of the nucleus locus coeruleus and descends through the caudal part of the pontine tegmentum mixed with the fibers of the lateral reticulo-spinal tract. At about the level that the facial nucleus appears fibers of the lateral tegmento-reticular tract have occupied a position somewhat medial to the ventral portion of the lateral reticulo-spinal tract and continue to descend in this position to terminate in the ventral medullary reticular formation in the region of the inferior olivary complex.

The efferent fiber connections of the nucleus locus coeruleus are thus limited to a descending homolateral lateral or ventrolateral tract of fibers which terminate in the ventromedial medullary reticular formation and which has been named the lateral tegmento-reticular tract.

In 1936 Kappers, *et al.*, suggested that the dorsolateral region of the superior reticular nucleus of the teleost, amphibian, reptilian, and avian forms is the primordium of the nucleus locus coeruleus of mammals. In the avian material that has been described in this communication, the owl and the robin, such a situation is evident and a nucleus which has the position of the nucleus locus coeruleus may be identified as a dorsolateral extension of the superior reticular nucleus. These observations add additional evidence to support the hypothesis that the nucleus locus coeruleus is a subdivision of the reticular nuclei of the metencephalic tegmentum and that in the course of phylogeny an anatomic separation of this nucleus from the superior reticular nucleus, the situation which occurs in mammalian forms, has occurred.

The earliest authors in general have left the implication that the nucleus locus coeruleus was a part of the trigeminal system. This has been denied by some and present authors are opposed to such an interpretation. There is no evidence for or against the thesis that it subserves some visceral sensory function although this has been suggested in the past. The third function of this nucleus, the proposal that it is a part of the respiratory mechanism, was first suggested by Lewandowsky (1904) and repeated

by Hess and Pollak (quoted from Finley and Cobb, 1940). Johnson and Russell (1952) confirmed this and identified the nucleus locus coeruleus as the classical "pneumotaxic center" described by Lumsden (1923).

As early as 1886 and 1890 Marckwald had demonstrated a respiratory center in the brainstem rostral to the medullary respiratory center. Lumsden (1923) named this center the "pneumotaxic center" and located it in the region of the upper pons. Stella (1938) confirmed the presence of some regulatory center for respiration in the upper pontine region and Pitts, *et al.* (1939) further localized it to the "tegmentum of the upper few millimeters of the pons." They stated that the red nucleus and the inferior colliculus were without this function, but that some center at this level gave rise to a homolateral descending pathway lying laterally or ventrolaterally in the pons and medulla and which influenced the activity of the medullary respiratory centers. Most recently Tang (1951, 1953) has described the "pneumotaxic center" as occupying a "discrete region in the dorsolateral portion of the anterior pons just caudal to the inferior colliculus," but he failed to name any particular nuclear mass.

Henderson and Sweet (1929) and Hess (1931) have maintained that the respiratory response elicited from the "pneumotaxic center" was the result of decerebrate rigidity following exclusion of the red nucleus and that the respiratory responses attributed to it were the result of a postural disturbance of the musculature of the chest rather than primary respiratory responses. Breckenridge and Hoff (1950) concluded that the primary regulation of respiration rested in the medullary respiratory centers and that secondary influences only were exerted by facilitatory and supressor regions of the brainstem above this level. This interpretation was amplified by Hoff (1954) to include the expiratory and inspiratory portions of the medullary respiratory centers themselves as functional subdivisions of the facilitatory and suppressor regions of the brainstem reticular formation.

That some structure in the region of the nucleus locus coeruleus exerts an influence on respiration seems, however, to be acceptable, although the name "pneumotaxic center" must probably be discarded as a misnomer. Keller (1929) reported respiratory alterations following lesions in this region and Johnson and Russell (1952) have described respiratory effects after lesions

localized to the nucleus locus coeruleus and after stimulation in this nucleus. Interpretation of the afferent and efferent connections of this nucleus can be made to conform to the hypothesis that the nucleus locus coeruleus is the classical "pneumotaxic center." A vago-locus pathway might supply information of a sensory nature from the lungs and respiratory passageways to the nucleus locus coeruleus. Trigemino-locus fibers can easily be understood as a part of the regulatory mechanism of respiration when one considers the importance of facial and masticatory musculature to respiration and articulation. The connection from the acoustic pathway proposed might serve to explain the sudden reflex inspiration observed in a "startle reaction" following a sudden loud sound. Collaterals from the dorsal longitudinal fasciculus and the suggested archistriato-mesencephalic homolog may deal with the cortical and hypothalamic regulation of respiration. That the latter pathway exists and does in fact influence respiration is to be deduced from the investigations of Kabat (1936) who reported an efferent respiratory pathway descending from the amygdaloid region to the pretrigeminal region in the position of the proposed archistriato-mesencephalic homolog. The descending efferent tract from the nucleus locus coeruleus confirmed in this communication and first described by Johnson and Russell (1952) as a respiratory pathway occupies the position predicted for such a tract by the neurophysiological investigations of Pitts, Ranson and Magoun (1936).

Some observations made by the author and by Johnson and confirmed by Olszewski (personal communications, 1951 and later) suggest that a broader interpretation of the functions of the nucleus locus coeruleus should be proposed. It is perhaps an oversimplification to assign only a respiratory regulatory function to this nucleus. Alterations of other vegetative functions; *i.e.*, cardiac and vaso-motor activity, micturation, and bowel control to name a few; have been observed after lesions and after stimulation in this region. Intimations that these changes have been observed by others is to be found in the literature, but as is often the case no careful record or anatomical study was made because it was not of primary concern at the moment. Certainly it would not be unexpected if several, or all, vegetative phenomena received some suprasegmental facilitatory or suppressor influences through a relay center such as the nucleus locus coeruleus.

Present emphasis on the importance of facilitation and suppression as functions of the brainstem reticular formation, and the now recognized widespread influence of this portion of the central nervous system strongly suggests that the nucleus locus coeruleus, which is part of the reticular formation, serves as a relay nucleus in the cortical and subcortical facilitation of central nervous control over general bodily vegetative functions.

VI. Summary

A detailed review of the literature and an extensive comparative anatomical study of the nucleus locus coeruleus are presented. This nucleus, found in all mammals, is composed predominantly of medium sized, round, oval, or fusiform neurons with rather deeply staining cytoplasm, frequently an eccentric nucleus, and a tendency for the Nissl substance to be clumped peripherally. Scattered throughout the nucleus are smaller, palely staining neurons of the type found generally in the central gray matter. The medium sized cells of adult primates, particularly man, contain finely divided granules of melanin pigment.

The nucleus locus coeruleus extends from about the level of the dorsal tegmental nucleus caudad to the rostral levels of the masticator nucleus. It is a prominent feature of the ventrolateral angle of the circumaqueductal and periventricular gray matter of the trigeminal and pretrigeminal tegmentum and invades, often deeply, the underlying reticular formation of the metencephalon. Comparative studies suggest that this nucleus is a derivative of the superior reticular nucleus and thus belongs to the group of reticular nuclei.

The afferent connections include: (1) fibers from the trigeminal nerve and its sensory nuclei, (2) collaterals from the neuropil which forms a continuous stratum in the ventrolateral angle of the periventricular gray over the entire extent of the IVth ventricle, (3) collaterals from the dorsal longitudinal fasciculus, (4) collaterals from the commissure of the lateral lemniscus, and (5) fibers from a suggested homolog of the archistriato-mesencephalic tract.

The efferent connections of this nucleus are limited to a descending homolateral lateral or ventrolateral tract which terminates in the ventral medullary reticular formation in the region of the inferior olivary complex. This confirms the description of

the lateral tegmento-reticular tract given by Johnson and Russell (1952).

The suggestion that the nucleus locus coeruleus is the classical "pneumotaxic center" of Lumsden is discussed, and it is proposed that this nucleus subserves a relay function in the cortical and subcortical facilitation of central nervous control over general bodily vegetative functions.

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A SCHEMATIC PRESENTATION OF THALAMIC MORPHOLOGY AND CONNECTIONS

GLENN V. RUSSELL*

In presenting any topic, regardless of the realm of knowledge under consideration, the lecturer is confronted with the problem of how much material the student is to be expected to learn and to understand. The presentation to medical students in neuroanatomy of the subject of thalamic nuclear morphology and fiber connections provides a clear-cut example of this dilemma. Certain basic facts of morphology and connections must be considered in order that the role of the thalamus in cerebral function can be discussed. The importance of these nuclei and their afferent and efferent connections in sensory and motor function are well known. Their more recent implication together with the "centrencephalic" system and the general problems of "consciousness" are of great importance and much current discussion. However, with probably no more than a single lecture to devote to the thalamus, and bearing in mind the true immensity of the subject which dictates a necessary restriction in the amount of detail that may be presented, some simplified but essentially complete and accurate schema is needed.

The author, therefore, has evolved during the last several years the schema which is presented here. Admittedly it retains several inaccuracies inherent in any schematic presentation. It has proven, however, to be a valuable teaching aid to the lecturer and a worthwhile and instructive study guide and mnemonic device to the student.

The morphological classification employed by the author and upon which the schema is in part based divides the dorsal thalamus into the following nuclear groupings:

A. Anterior Nuclear Group
   1. Nucleus anterior (R)
   2. *Nucleus anterodorsalis* (R)

B. Medial Nuclear Group
   1. Nucleus medialis dorsalis

* From the Department of Anatomy, The University of Texas Medical Branch, Galveston. Illustrative expenses defrayed by USPHS–NINDB Grant B–751. Submitted for publication July 8, 1955.

a. pars parvocellularis (A)
b. *pars magnocellularis* (?)

C. Midline Nuclear Group
1. *Nucleus parataenialis* (?-D)
2. *Nucleus paraventricularis* (?-D)
3. *Nucleus reuniens* (?-D)
4. *Nucleus rhomboideus* (?-D)

D. Intralaminar Nuclear Group
1. *Nucleus centrum medianum* (D)
2. *Nucleus parafascicularis* (D)
3. *Nucleus paracentralis* (D)
4. *Nucleus centralis lateralis* (D)
5. *Nucleus centralis medialis* (D)

E. Lateral Nuclear Group
Pars dorsalis
1. Nucleus lateralis (posterior) (A)
2. Nucleus lateralis dorsalis (A)
3. Pulvinar (A)
Pars ventralis
4. Nucleus ventralis anterior (R and D?)
5. Nucleus ventralis lateralis (R)
6. *Nucleus ventralis ventralis* (R)
7. Nucleus ventralis posterior (R)
a. *pars lateralis*
b. *pars medialis (arcuatus)*

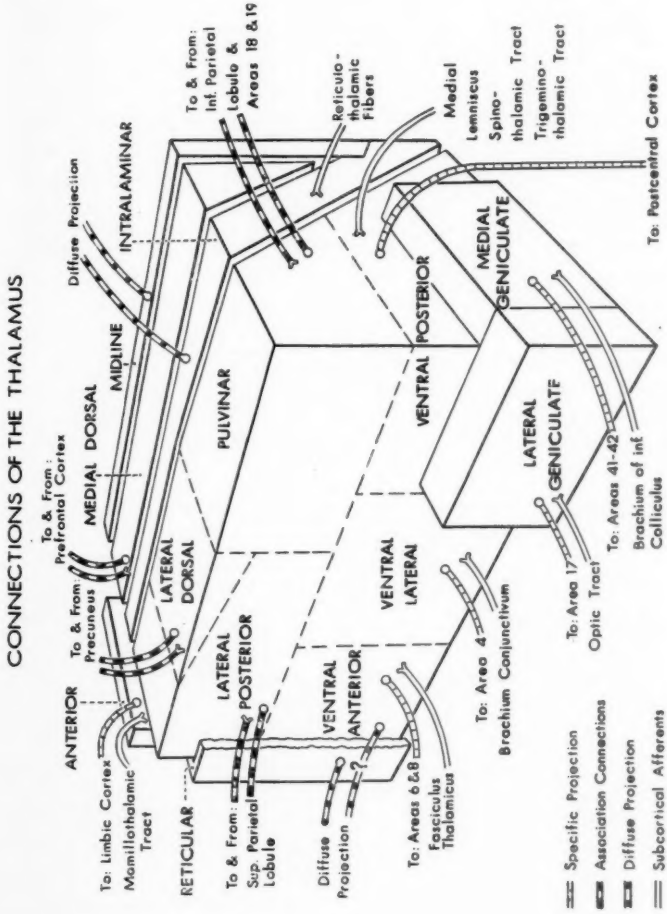
F. Metathalamus
1. Corpus geniculatus laterale (R)
2. Corpus geniculatus mediale (R)

G. Miscellaneous unclassified nuclei
1. *Nucleus submedius* (?)
2. *Nucleus subgeniculatus* (?)
3. *Nucleus limitans* (?)
4. Nucleus reticularis thalami[1] (D?)

Those nuclei which normally are considered as a part of the instruction on the functional systems are set in roman type while those less prominent and usually unmentioned nuclei are set in italic type. The major nuclei of the dorsal thalamus are presented individually in the schema while the nuclei of the intralaminar and midline groups are presented collectively. In the schema all of the nuclei of the dorsal thalamus are presented as forming essentially a block which has been subdivided in simple planes to portray the general spatial relationships that exist between individual nuclei and groups of nuclei.

[1] The nucleus reticularis thalami is properly a nucleus of the subthalamus rather than of the dorsal thalamus, but is included here by tradition and general usage.

The principal afferent and efferent fiber connections for each of the nuclei or groups of nuclei are indicated in the schema. Certain generally recognizable similarities in the pattern of connections permit a functional classification of thalamic nuclei as follows:



I. Nuclei with Specific Relay Connections—(R).
II. Nuclei with Specific Association Connections—(A).
III. Nuclei with Diffuse Cortical and/or Subcortical Connections—(D).
IV. Nuclei in doubt or as yet unclassified—(?).

Each of the nuclei listed above has been placed in what would appear at the present time to be its correct functional grouping by the appended parenthetical designation. The intrathalamic connections which must exist in abundance are so poorly understood that they must be omitted for the present. In the schema the functional grouping of nuclei according to the pattern of their afferent and efferent connections is indicated. The schema thus presents succinctly a large part of the fundamental information of the morphology, connections, and organization of the dorsal thalamus in a highly simplified form.

CANCER OF THE LUNG BEFORE 1900: A HISTOLOGIC REVIEW*

R. H. Rigdon

A statement by Stewart in 1954 indicating the confusion referable to the early observations on cancer of the lung served as the stimulus for me to review the problem. The observations made before 1900 are important in evaluating this lesion today. The earliest records of cases, subsequently accepted as probably pulmonary cancer, are present in the writings of Agricola in 1521 to 1527, Pansa in 1614, and Engelschall in 1725 (Simons, 1937). Van Swieten's Commentaries published in 1747 cite a case of a 50-year-old man whose "right lung was found scirrhous in its upper part, thrusting the mediastinum and esophagus towards the left side, and so much compressing the cavity of the esophagus, that the tube appeared greatly dilated above the obstruction." This, of course, may have been a primary carcinoma of the lung but we have insufficient data to warrant such a diagnosis. In 1761 Morgagni described the case of a "cancerous ulcer" in the lung of a patient 66 years of age. Stokes (1844) in discussing it said, "The details of the case are extremely scanty" and apparently did not accept it as an authentic case of cancer of the lung.

Bayle in 1810 reported three cases of cancer of the lung, two of which were metastatic. The third has been accepted (Stokes, 1844; Adler, 1912; Simons, 1937) as the first reported case of primary lung cancer; it occurred in the year 1805 in a man 72 years of age who "had enjoyed good health until within six weeks of his admission into the hospital. His disease set in with pains affecting the whole body, but principally engaging the chest and epigastrium. A slight cough, with white opaque expectoration, set in, the appetite failed, and the bowels became obstinately costive; the liver was enlarged and irregular, filling the epigastrium, and extending almost to the umbilicus. Three hard, indolent, moveable bodies, of about the size of nuts, were found to exist in the epigastric and right hypochondriac regions." At

* From the Department of Pathology, The University of Texas Medical Branch, Galveston. This is part of a cooperative study with the Medical College of Virginia.

autopsy "the root of the left lung was found occupied by a mass of shining, white appearance, in the interior of which, red capillary vessels could be seen. In the centre of this substance, which resembled brain, and also in the lung itself, tuberculous masses could be detected, easily distinguished from the cancerous matter by their yellow, opaque appearance; several small tuberculous cavities were found in the remainder of the lung. The liver contained numerous cerebriform masses, and the moveable subcutaneous bodies were evidently of the same nature as the internal tumors" (Stokes, 1844).

One of the first cases of primary cancer of the lung reported in America was published in 1851 by Storer. The patient was a male 39 years of age. He had an attack of pleurisy in the spring of 1850 and died the following September. He had a cough that was accompanied by expectoration of "adhesive mucus" and suffered more or less constantly from dyspnea. At autopsy an encephaloid mass occupied more than the lower two-thirds of the right lung. "In right primary bronchus, disease had extended upon the inside within half an inch of bifurcation of trachea, reducing the bronchus to one-half of its ordinary size, and the cartilaginous ridges on its cut edge were seen to be embedded in the encephaloid matter. A large mass existed behind the trachea in the cellular membrane which invests the trachea and large vessels. In the midst of the disease were found several hardened glands, much enlarged and infiltrated with encephaloid matter. No disease discovered in other organs."

Passler in 1896 reviewed the problem of cancer of the lung and stated that "the oldest statement known to me on the finding of cancer of the lung is by Lieutaud (1767) . . . The oldest data on primary cancer of the lung seems to go back to Haase (1841) who knew of a primary myeloid-spongy degeneration of the lungs." Although 132 cases of primary cancer of the lung had been collected from the literature by Passow (1893), Dorsch (1886), Hildebrand (1888), and Wechselmann (1882), Passler (1896) accepted only 70 since "no cases without a post mortem report can be considered."

Interest in cancer of the lung was not restricted to human cases before 1900. Siegert in 1893 referred to cancer of the lung in a dog. The neoplasm was a "Cylinder-cell cancer" and involved one lower lobe of the lung. There were no metastases.

Frequency of Cancer of the Lung. Adler (1912) found 211

reported cases of cancer of the lung before 1900, five of which were observed between the years 1825 and 1849, 31 between 1850 and 1874, 64 between 1875 and 1889 and 111 between 1890 and 1900. Within corresponding years 65 cases of sarcoma of the lung were reported (Adler, 1912); one of which was observed between 1825 and 1849, six between 1850 and 1874, 25 between 1875 and 1889 and 33 between 1890 and 1900. Rokitansky (1854) considered cancer of the lung to be exceedingly rare. However, in England, Stokes (1837) had this to say, "Lung tumors are by no means as rare either in England or in Ireland as was generally assumed."

At the Dresden General Hospital cancer of the lung steadily increased in frequency from 1852 to 1885 (Wolf, 1895). During this period 45 cases of pulmonary cancer were found in 20,116 autopsies. It is of interest to note, however, the progressive increase in the frequency of this lesion during this period: 1852 to 1876, 0.057 per cent; 1877 to 1884, 0.21 per cent; 1885 to 1894, 0.428 per cent. Fuchs (1886) found eight cases of primary pulmonary cancer in 12,307 post mortem examinations at Munich Pathologic Institute between the years 1854 and 1885. This was 0.065 per cent. Reinhard in 1878 found cancer (carcinoma and sarcoma) 545 times in 8,716 autopsies. Among these were five cases of primary and 74 cases of metastatic lesions in the lung. Passler (1896) observed 870 carcinomas and 130 sarcomas in 9,246 autopsies between 1881 and 1894 at Breslau. The following were the most frequent primary sites: stomach, 319 cases; female genitals, 197; esophagus, 83; breast, 59; intestines, 56; gallbladder and large bile ducts, 43; lung, 16; liver, 13. Primary cancer of the lung was not considered by Passler (1896) to be rare in Breslau since it amounted to 1.83 per cent of all the carcinomas and 1.6 per cent of all malignant tumors. Wolf (1895) said that "primary pulmonary carcinomas are more frequent in our town than elsewhere."

Today there would be disagreement on some of the early diagnoses; however, these data do indicate the trend with regard to the increased frequency in the occurrence of primary neoplastic lesions in the lung before 1900. There were many children and young adults in the group of so-called sarcomas of the lung; no doubt today many of these would be classified as malignant lymphomas and metastatic neoplasms.

As one would expect, the increase in the frequency of intrathoracic cancer before 1900 was accompanied by a corresponding increase in all cancers. In England and Wales in 1840 (Williams, 1908) the proportion of cancer deaths to total deaths was one to 129 and in 1900 it was one to 22. This increase in the frequency of cancer was attributed by some to an increase in the average age of the population; however, Williams (1908) emphasized the fact that the population barely doubled between 1850 and 1905, but the cancer mortality increased more than sixfold. King and Newsholme (1893) expressed the opinion that this increase in the frequency of cancer was only apparent, being due to improved diagnoses and more careful certification of the causes of death.

The percentage of intrathoracic cancer referable to all neoplasms, as determined from the autopsy before 1900, has been studied by Bonser (1928). It is of interest to observe the variations in the frequency of these neoplasms in different cities: Hamburg, 1894–1898, 1.95 per cent; Prague, 1895–1899, 2.62 per cent; Manchester, 1886, 1.58 per cent; and Leeds, 1893–1897, 7.42 per cent. Duguid (1927) found an increase in intrathoracic cancer at Manchester from 1.58 to 2.57 per cent between the years 1886 and 1925, while Bonser (1928) found no increase in the frequency of this lesion at post mortem at Leeds between 1891 and 1927. Bronchogenic carcinoma was not specifically classified and recorded as such in the vital statistics of the United States until 1939 (Gilliam, 1955).

Etiology of Cancer of the Lung. The attempt to establish the etiology of lung cancer was as difficult before 1900 as it is today. The relationship of tuberculosis to pulmonary cancer first arose in 1810 following publication of the case by Bayle (1810). "It is known that in earlier times Rokitansky denied the existence of tuberculosis and carcinoma in one and the same body and even insisted that one of these neoplasms produced a poison which inhibited the growth of the other, the observation has been made very frequently recently that carcinoma and tuberculosis do exist in the same individual and that tuberculosis forms a base on which the carcinoma prefers to settle" (Wolf, 1895). Crazet in 1878 expressed the opinion that "the cancerous easily becomes tuberculous but the tuberculous do not easily become subject to cancer." Thirteen of the 31 cases of pulmonary cancer reported by Wolf (1895) were complicated by tuberculosis. Hampeln

(1897) agreed that tuberculosis and carcinoma could occur simultaneously.

The role of chronic inflammation in the etiology of cancer of the lung was discussed by Bayha in 1888. Wolf in 1895 stated that "Virchow's assumption that carcinomas develop mainly at those places which have been exposed to an intensive irritation for a rather long time will have the greatest number of followers. But no irritation is of longer duration and larger than that caused by scars of any type. For this reason attention has been focused on the formation of cancers from scar tissue." Wolf (1895) cites the frequency in which small fistulous canals accompanied by black pigmentation occur in the bronchi. "The cause of this pigment perforation is a softening of an earlier enlargement and a hardening of the bronchial glands which were colored black by dust and carbon particles. The enlargement and hardening of the latter represent the final products of inflammatory processes of many types which have taken place there and among which tuberculosis is of the greatest importance." Wolf (1895) also commented on the fact that "chronic colds, ozena, etc. . . . also change the cylindrical epithelium of the nasal mucous membrane into pavement epithelium from which it is possible that a typical squamous cell epithelioma can develop." Four cases of lung cancer attributed to trauma were collected in 1897 by Perutz.

The pulmonary lesion so frequently found in the miners at Schneeberg in Germany and Joachimstal in Czechosolvakia was first studied extensively in 1879 by Härting and Hesse. They considered it to be a lymphosarcoma originating at the root of the lung; however, in 1913 Arnstein considered the same lesion to be a carcinoma. Considerable discussion has centered around the etiology of this pulmonary lesion. Recently Lorenz (1944) reviewed this problem without arriving at a specific etiology. This author (Lorenz, 1944), however, does not consider that radon was the sole cause of these bronchogenic neoplasms. Pneumoconiosis produced by the dust in the mines, chronic irritation caused by respiratory diseases, arsenic, radioactive substances, and heredity must be considered as possible contributory factors to the etiology of this neoplasm.

Langstaff's (1818) theory for the origin of pulmonary tumors is worthy of note. He had noticed "pulpy tumors in the lungs of adult persons, who had not been affected during their lives with

the least symptoms of pulmonic disorder, and who died of active disease of a different description in other viscera." He was inclined to think that "these specific and malignant diseases such as cancer, Fungus Hematodes, and scrophula have their origin perhaps with the formation or development of the natural parts of the foetus in utero; and that they remain, after the birth of the individual, in some instances dormant or inactive for a series of years, and in all only require a peculiar morbid excitement to occasion this increase and destructiveness." A somewhat similar idea was suggested by Ernst in 1896; he was impressed by the frequency in which these neoplasms occurred at the hilus and attributed them to the development from a germinal remnant.

According to John Marshall (1889) all cancers arose from "complete anarchy." This anarchy was due to lack of nerve influence. A few years later Beneke (1892) pointed out that the nervous system can only regulate the forces contained in the cells and suggested a disturbed equilibrium in the relation and proportion of cell function as a causal factor for the etiology of tumors. Boyd (1887) concluded from his clinical study that "primary cancer of the thorax is of not unfrequent occurrence, generally appearing in hard drinkers, and the symptoms of it are developed apparently after catching a cold." William Osler (1892) wrote that primary tumors of the lung were rare. They were either encephaloid, scirrhous, or epitheliomatous. Primary carcinoma usually involved only one lung. The disease was common in the middle period of life and affected the sexes equally. Cancer of the lung had been recognized by Walshe (1871) to be more frequent in the male. West (1897) gave a ratio of three males to one female. He cites an observation by Haase in which the ratio was four to one, and another by Kohler which was five to three. In Fuchs's cases (1886) there was 38 males and 26 females. Wolf (1895) had 27 males and four females, and Passler (1896) had 50 males and 18 females.

After carefully examining the Vital Statistics for England and Wales for the latter half of the 19th century, Williams (1908) concluded, "The cancer mortality is lowest when the struggle for existence is hardest . . . the average duration of life shortest . . . and where sanitation is least perfect—in short, among the poor of the industrial classes in our large towns, whereas, among the wealthy and well-to-do . . . the cancer mortality is highest . . .

there are, in my opinion, no more potent factors in the causation of cancer than high feeding and easy living."

Pathology of Lung Tumors. The case of cancer of the lung reported by Bayle in 1810 emphasized the fact that these neoplasms occurred at the root of the lungs and that their gross appearance suggested brain tissue—"white encephaloid cancerous masses." As late as 1872 Jaccoud stated that primary cancer of the lung usually was of the encephaloid variety, and that it occurred in two forms, a single mass and a diffuse form, the former being more frequent. The term "encephaloid" was retained for these pulmonary neoplasms until Aschoff devised the following classification: (1) small nodular masses arising from a bronchus of the first to third order and (2) the infiltrating type in which a large portion of the lung or an entire lobe is involved. Kaufmann added a third type, a circumscribed tumor located in the midst of a lobe. Wolf (1895) called attention to the fact that pulmonary cancer developed anywhere in the lung, while bronchial carcinoma regularly developed "immediately below the bifurcation in one of the two principal bronchi." In 1880 (Cornil and Ranvier) it was pointed out that pulmonary cancer was found more often in the right lung than in the left. Cavities in cancerous nodules in the lungs were commented upon by Jaccoud in 1872 and by Cornil and Ranvier in 1880. Apparently they were rare. Arnal in 1844 found such a lesion in a 64-year-old man with a tumor in the right lower lobe obstructing the right main bronchus. Similar cases were reported by Auvard in 1882 and by Salter in 1869.

Langhans in 1871 pointed out that primary cancer of the trachea was one of the greatest rarities and quoted Förster as saying "they have not yet been observed." Langhans (1871) reported a case occurring in a male about 40 years of age who suffered for approximately one year from stenosis of the bronchi. At autopsy the lower 4.0 cm. of the trachea and the adjacent bronchi were involved by a neoplastic process. "The strongest and most heterogeneous swelling of the mucosa occurs exactly at the point of division of the trachea and the initial parts of the large bronchi. Here occur, on the posterior and lower side of the trachea, hemispherical smooth or slightly rough nodules as much as 7 mm. in height, which rest upon a broad or somewhat constricted base and extend into the right bronchus 2.5 cm. farther. Moreover, the orifice of the right bronchus is also greatly constricted so that

on the other sides of its wall, on the uniformly swollen mucosa, there rest a series of smaller verrucose elevations which in connection with those larger nodules surrounds its lumen annularly and constricts the latter." The microscopic findings were "entirely that of the soft cancers of the mucosa . . . the mucous glands can be recognized as the point of origin of the entire cancerous structure." Langhans (1871) gave a beautifully detailed discussion of the pathogenesis of this neoplasm from the mucous glands of the bronchial mucosa—"the processes which lead immediately to the formation of the cancerous structure, with complete disappearance of the glandular arrangement, are the disintegration of the enlarged terminal vesicles into the individual columns of cells and the growing out of the latter into the surroundings. We must consider these two processes with the disappearance of the membrana propria as essential ones which characterize the entire disease."

Some of the early macroscopic descriptions of cancer of the lung are most interesting. Cases were described in which the neoplasm extended into the mediastinum and produced paralysis of the vocal cords (Wolf, 1895; Boyd, 1887; Arnal, 1844), invaded the pericardium (Ehrlich, 1882; Auvard, 1882) and heart (Hofmann, 1893; Ehrlich, 1882), constricted the major blood vessels (Boyd, 1887; Arnal, 1844; Jaccoud, 1872), and invaded the wall of the vena cava (Walshe, 1871; Beck, 1884). Metastases were described in the brain (Wolf, 1895; Ernst, 1896; Walshe, 1871; Chiari, 1883), ribs (Ehrlich, 1882; Holye, 1883), femur (Wieber, 1889), vertebrae with compression myelitis (Ehrlich, 1882; Holye, 1883), liver (Dorsch, 1886; Chiari, 1883; Finlay and Parker, 1877), spleen (Dorsch, 1886; Chiari, 1883; Japha, 1892), kidney (Dorsch, 1886; Holye, 1883; Werner, 1891), thyroid (Beck, 1884; Siegel, 1887; Ehrlich, 1882), adrenals (Ernst, 1896; Stilling, 1881; Beck, 1884; Siegel, 1887), and muscles of the skeleton (Handford, 1888; Wolf, 1895).

The histologic site of origin of pulmonary cancer was discussed frequently before 1900. Chiari in 1883 suggested that adenocarcinomas originated from the bronchial mucous glands. In 1895 Wolf suggested that such tumors may also arise from the bronchial mucosa. The origin of squamous cell carcinoma in the lungs presented a problem. Wolf (1895) stated that "those carcinomas which originated from the alveolar epithelial naturally must be squamous-cell epitheliomas since the alveola are nor-

mally covered with pavement epithelia. . . . Squamous-cell epitheliomas originating from alveolar epithelia are very rare. Grunwold (1889) who personally examined such a tumor in great detail, only found two similar cases in the literature." Similar cases subsequently were reported (Ernst, 1896). Twelve of the 16 cases of lung cancer reported in 1899 by Fröhlich were of the "pavement epithelium."

A major problem arose in the pathogenesis of pulmonary tumors when squamous cell carcinomas were found originating within the larger bronchi. In 1895 Wolf said, "The method of development of squamous-cell epitheliomas on the mucous membranes of bronchi usually covered by a ciliary epithelium requires a short explanation." He discussed the fact that metaplasia in the air passage had been described previously in chronic irritation. Griffini in 1884 apparently was one of the first to state that "in the course of tuberculous processes mucous membranes are capable of changing their normal covering of ciliary epithelia into one with pavement epithelia. . . . It is comprehensible that with such continuous irritation and under such conditions the newly-formed pavement epithelium can be excited to atypical proliferative processes" (Wolf 1895). In 1896 Ernst discussed in an excellent manner the theory that squamous cell carcinomas may arise as the result of metaplasia. Siegert (1892) described a benign papilloma with epithelial pearls in the trachea near the bifurcation which he considered to indicate a metaplastic change.

Passler (1896) discussed at length the histogenesis of bronchogenic carcinomas referable to their origin from (a) bronchial covering epithelium, (b) mucous gland epithelium, and (c) alveolar epithelium. He pointed out "how contradictorily most of the findings are interpreted by the various authors. . . . Most of the lung cancers, according to the present state of our knowledge, do not permit a definite histogenetic judgment. Histogenic probability diagnoses are, however, of little value for our knowledge."

In the theories on the origin of lung cancer it apparently has been overlooked that all parts of the lung arise from the same primitive bud. The mucous glands, therefore, have the same origin as the bronchial epithelium. It is not unusual to note that many pathologists, from 1810 to the present, find it difficult to classify satisfactorily all bronchogenic carcinomas on a histologic basis.

Cytologic Examination of Sputa for Cancer Cells. Walshe in 1871 pointed out the fact that cancer cells occasionally had been found in sputa. Hinterstoisser (1889) made an examination of the sputum for tumor cells from a male 59 years of age, who subsequently died with a carcinoma of the trachea and bronchi. The sputum was mucoid and often bloody. It contained numerous epithelial cells single and adherent to each other. Another interesting case using this technique was reported by Sormani in 1890. The patient was a 26 year old female who observed a "black hair" in her sputum. At autopsy a dermoid tumor filled with yellowish-gray, creamy, atheromatous material of nauseating odor, containing hair, was found in the left lung. Ehrlich (1891) reported a case of a male 51 years of age who expectorated bloody masses which contained cancerous material. At autopsy there was a cancer of the right bronchus. In 1899 Pearson, in North Carolina, suspected a malignancy when a mass one by one-half inches was coughed up by a forty-one year old male. The patient had tuberculosis and organisms were demonstrated in the sputum. An autopsy confirmed the diagnosis of "encephaloid carcinoma and tuberculosis." In another case in 1899 (Claisse) a male 50 years of age expectorated two particles about the size of a cherry pit; one of these was prepared for histologic study and showed an epithelioma.

Hampeln in 1897 reviewed the problem of "the expectoration in lung cancer" and reported his observations on both the macroscopic and microscopic characteristics of the sputum. "I believe that it is right that emphasis be placed not on the hemorrhagic character of the sputum, but on a characteristic color. This limitation, although not of positive pathognomonic value, appears to be one of the most important criteria, since it often makes it possible to distinguish immediately or after a short observation period between this disease and the principal concurrent ones, such as chronic tuberculosis, lung infarct and croupous pneumonia. . . . There is usually a mild bleeding, easily overlooked, passing quickly, and repeated two or three times. . . . All in all this hemmorrhagic nature of the expectoration, its viscous consistency, and its temporal behavior, can be considered as an important and noteworthy sign . . . lung hemorrhage is an ambiguous phenomenon in and of itself, and certainly has no significance for differential diagnosis, but if properly observed as an important warning signal, it can make a contribution to the

differentiation, if only by awaking the perhaps otherwise omitted suspicion of a neoplasm." Greater attention was given to the microscopic examination of sputum by Hampeln (1897) in the diagnosis of lung cancer. Following a discussion of the normal cells originating in the bronchial tree and "metamorphosed leucocytes or wandering cells," such as are so frequently found in heart failure, infarcts, miner's lung, pharyngeal mucus of smokers, and even in individuals without signs of bronchial or lung disease, Hampeln (1897) concluded that "now if one finds, even for one day, in the sputum of a patient, exceptionally or predominantly, such cells that are easily distinguishable from pavement and cylindrical cells: pigment-free polymorphous polygonal cells of different sizes, with clear nucleus contours and clear nucleolus, in clusters or separate, some of them giant cells, one would be entirely justified in reaching the conclusion that *there is a neoplasm producing these forms which would not otherwise be present*."

Passler in 1896 emphasized the significance of examination of sputum by saying "in all cases of very stubborn and especially narrowly circumscribed bronchitis, to examine the sputum in a fresh condition repeatedly and most carefully, even if microscopically no peculiarities are noticed."

Ehrlich in 1882 called attention to the diagnostic value of cell groups in pleural effusions in the diagnosis of cancer. Hellendall (1899) utilized this technique in a 47 year old patient who had a bloody effusion in the right chest. This fluid showed "heaps of round cells from which the diagnosis of sarcoma of the lung was made." At autopsy a typical sarcoma was found in the right lung. One of the first known needle biopsies of the lung was made in 1887 and a diagnosis of lymphosarcoma was made from the specimen (Krönig, 1887).

Clinical Observations on Cancer of the Lung before 1900. In a paper such as this, one cannot adequately discuss the clinical observations made on cancer of the lung before 1900. However, it is most stimulating to read the contributions made on this subject by physicians at a time when the techniques for physical examination, ausculation, and precussion had to be used for diagnosis rather than the bronchoscope and roentgenograms.

The discussion of Graves (1884) following a postmortem examination on a case of lung cancer which he had failed to diagnose is most enlightening: "It is scarcely necessary to observe to

you (students) that both Dr. Stokes and myself were completely mistaken as to its nature. . . . I became quite tired of the difficulty of attempting to explain the phenomena observed, with any of the diseases I had originally fixed on as the cause of the symptoms; and latterly, however, erroneously positive I had been when I took the man under my care, I gave up all further attempts at diagnosis; and yet it seems strange that the external tumors (metastases in the skin) did not awaken a suspicion of the true nature of the case, for although we were not permitted to examine them, their nature was certainly the same with the internal. The truth is, that these very tumors served only to mislead me still further, for I considered them as common scrofulous formations."

Graves (1884) emphasized that cancer of the lung was an uncommon disease. However, he warned his students that "rare diseases should not be looked upon as mere matters of curiosity, but should be attentively studied with the view of enabling us to recognize the true nature of similar cases when they again occur. . . . The diagnosis of encephaloid tumors of the lungs was, a few years ago, completely impossible; but I trust that ere long we may be enabled to arrive at some degree of certainty even in this difficult and obscure branch of thoracic pathology."

The clinical diagnosis of cancer of the lung, as related by Stokes in 1842 could not be positively made: "Cancerous disease of the lung is met with in two forms; in the first, a degeneration of the lung occurs, and the organ is transformed into a cancerous mass without the production of any tumour. In the second, the scirrhous or encephaloid matter forms a tumour, at first external to, and ultimately displacing the lung. In neither case can we apply any direct diagnosis; and I do not know how the first could be determined with certainty. The symptoms are always obscure, and the physical signs being merely those of solidity, more or less extensive, the greatest difficulty exists in making an accurate diagnosis. Repeated observations, indeed, might lead us to doubt whether the lesion was an ordinary disease, and the existence of external cancer could give a probability, that the internal affection was of the same nature. But, in a case seen for the first time, and in which no external disease existed, we have no means by which its nature could be positively determined." Salter (1869) in reviewing a case with his students emphasized the fact that "there can be no doubt that the cancerous growth existed

some time before the patient was aware of it. . . . This is just what we see in cases of primary cancer of the lung; and it is sometimes surprising how far the disease may advance without revealing itself by what would be thought likely to be its characteristic symptoms."

Sputum was considered by some as very significant in the diagnosis of cancer of the lung. Stokes (1844) was one of the first to describe sputum in a case of cancer of the lung as like "black currant jelly." Elliott (1874) described it as olive grass-green; however, Passler (1896) considered grass-green sputum associated with sarcoma. West (1897) expressed the opinion that "expectoration is not constant. . . . It may be absent even when perforation has taken place owing to the obstruction to the air-tubes, unless the perforation be on the tracheal side of the tumor. The sputum may be catarrhal in character, *i.e.*, muco-purulent, hemorrhagic, or contain some of the new growth or fragments of lung tissue." Walshe (1871) stated that in cancer of the lung less than one-third escape haemoptysis altogether. Hampeln (1897) concluded that in cancer of the lung there was usually mild bleeding but abundant hemorrhages were rare.

Passler, in an excellent article published in 1896, reviewed 70 cases of cancer of the lung. After establishing the most frequent pathologic characteristics, he discussed the clinical signs and symptoms based upon these cases. It was pointed out that clinical signs and symptoms were frequently absent in cases of lung cancer until metastases occurred in vital organs. "If there are no metastases present which lead to clinical symptoms then the sickness may exist for months . . . without cachexia or the local progress of the process directly to death."

Contributions made by the stethoscope to the diagnosis of lung cancer were numerous between 1800 and 1900. Behier (1867) described a peculiar sound over the trachea in cases of cancer of the lung to which he gave the name "cornage." He called attention to the fact that this may be a very early manifestation of pulmonary cancer. Woillez (1870) designated as characteristic of lung tumors what he called the "tympanisme thoracique" which, according to Adler (1912), consisted of a tympanitis—immediately preceding the full—percussion note. Observations on auscultation as made before 1900 have been discussed in the monograph by Adler (1912).

Boyd (1887) gave an excellent discussion of the different diagnosis of carcinoma of the lung in an article published in 1887 emphasizing the effect of pressure on the structures in the mediastinum, such as veins and laryngeal nerves with paralysis of the vocal cords. With regard to tuberculosis, Boyd (1887) said: "Though the symptoms of cancer of the bronchial glands resemble phthisis very closely, we shall find in signs occasionally features that raise doubts in our minds as to their being phthisical ones. . . . When the bronchial glands are much enlarged and compress the bronchi, vocal fremitus and vocal resonance are both absent; and absence of all breath sounds with collapse or consolidation of the lung generally present a condition you will not find in phthisis, no matter how extensive the consolidation."

Walshe (1871) emphasized the following diseases in the differential diagnosis of cancer of the lung: cirrhosis, tuberculosis, chronic pneumonia and chronic pleurisy. He did not think either arsenic or a change of climate beneficial in the treatment of this lesion. The prognosis was absolutely fatal. "I have no evidence that the disease ever undergoes spontaneous suspension of course much less arrest."

It has been 118 years since Heyfelder (1837) became so dissatisfied with the treatment that patients with cancer of the lung were receiving, under all sorts of diagnoses, the blood-letting, the purging, the salivation, that he urged physicians to realize the necessity of recognizing these cases of cancer of the lung as hopeless and begged them not to add the torture of medical treatment to the sufferings consequent upon the disease itself.

Medicine since 1900 has contributed little to the basic knowledge of the histogenesis of lung cancer. Considerable advancement, however, has been made in the roentgenographic diagnosis of this disease. The problems as to how early diagnoses may be made are still to be solved. Whether the increase in the frequency of cancer of the lung is real or only apparent is still to be determined. The need for greater emphasis on this disease in our undergraduate and graduate programs is as necessary today as it was emphasized to be more than 100 years ago.

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LEONARDO DA VINCI AS ANATOMIST AND PHYSIOLOGIST: A CRITICAL EVALUATION*

J. B. DE C. M. SAUNDERS

To the universal genius of Leonardo da Vinci, all the world attests. At the end of the half millenium which lies between his birth and the present, he has grown in stature. Leonardo's achievements as an artist were fully recognized by his contemporaries who ranked him with the masters and their judgment is confirmed by the present, but full recognition of his merits as a scientist has had to await the appreciation of our own mechanical age. This dual recognition, which places Leonardo in the first rank among both artists and scientists alike, makes him unique in the history of western culture and testifies to the astounding universality of his mind. The union of the great observational powers and talents of the artist with the penetration and comprehensiveness of the scientific mind was perhaps the major source of his genius and nowhere in his works is this more apparent than in his investigations into the structure and function of the human body. Here within the confines of a single discipline it is possible to observe the great power of his artistic eye and hand in the recognition and recording of human structure combined with his insight into the natural sciences as applied to the solution of functional problems.

In paying tribute to Leonardo's astounding achievements we should be fully aware of his limitations and his weaknesses. It is understandable that the universality of his genius should inspire wonder and awe, but at the same time this should not deprive us of critical judgment. To fully appreciate Leonardo he must be viewed against the background of his contemporaries. We must examine his personal development, compare his sources, follow his influence and record not only his positive contributions but also his failures. It is regrettable that this neglect to take a more comprehensive view of Leonardo's achievements has been responsible, especially in the field of anatomy and physiology, for a vast number of spurious claims on his behalf such as the fre-

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quent assertion that Leonardo before Harvey was "the first to understand the circulation of the blood" or that he was "the first after Aristotle and Galen to revive the comparative method" in anatomy. These false assertions and unjustifiable claims do little to help our understanding of Leonardo and his difficulties, nor do they provide insight into the factors limiting the progress of science.

Two factors have been largely responsible for the false estimates of the Vinician contributions to anatomy. These are the failure to give serious consideration to Leonardo's notes and the too ready acceptance of his illustrations at face value.

Haphazard and disorganized though his writings may be, without them it would be impossible to perceive the groping of Leonardo's mind as he sought emancipation from a debased mediaeval Aristotelianism and corrupted Galenism to a position of relative scientific independence. Incidentally, those unfamiliar with the peculiarities of Leonardo's language and nomenclature are not helped by the atrocious quality and inaccuracies of most of the modern translations of his notes. Without the notes the dynamic and functional aspects of his work are lost almost completely, since so many of his illustrations are no more than diagrams to clarify the text and must not be interpreted as factual morphological representations.

It has been repeatedly stated that Leonardo was little influenced by contemporary and traditional sources owing to his deficient knowledge of the classical languages. This is by no means true as may readily be determined by his own writings. To the end of his days Leonardo carried an oppressive load of barren learning and authoritative error which time and time again diverted him when his approach would seem to have brought him to the very brink of a major discovery.

The graphic arts are of immense power in the biological sciences and Leonardo's drawings excel by far anything that had preceded them. Yet by their very power they have proved misleading for too often they are deliberate distortions, although highly naturalistic in appearance, of animal anatomy to the form and the proportions of man. Often they represent beliefs rather than the actual findings in nature and in so doing they testify that Leonardo, like his contemporaries, was unable to avoid that excessive reverence for the written word which was so stultifying to scientific development.

The difficulties encountered by Leonardo in his approach to the fields of anatomy and physiology were two-fold; first, an attitude of mind and second, an undoubtel scarcity of human dissection material.

For Leonardo, as it was for almost all biologists up to the middle of the nineteenth century, the disciplines of anatomy and physiology were one. A teleological tradition extending back to the days of Galen could countenance no separation of structure from function. Thus we find the great majority of Leonardo's anatomical drawings are not simple statements of morphology, but are at the same time representations of function or purpose. Not infrequently it is the presupposed function, in the mind of Leonardo, which determines the drawing of the anatomical structure and not its appearance as revealed by dissection. Leonardo could not escape his teleological preconceptions.

The amount of human material available to Leonardo was strictly limited. In his early or first Milanese period circa 1481–1499 Leonardo depended almost entirely upon animal dissections with an occasional part from the human subject. During his Florentine and second Milanese period, opportunities for human dissection would seem to have been more plentiful, but never great. After 1510 he was compelled to revert once more to animal dissection. Around the year 1510 Leonardo stated that he had dissected "more than ten bodies"[1] since he had had to proceed by degrees owing to the lack of preservatives. This estimate is doubtless correct as judged from the internal evidence of his writings, but at the same time it should be recognized that these were probably incomplete dissections for, as he himself says, "A single body was insufficient for so long a time." It is not unlikely that at some of these dissections he was no more than a spectator. Consequently he was compelled to fall back upon the butcher

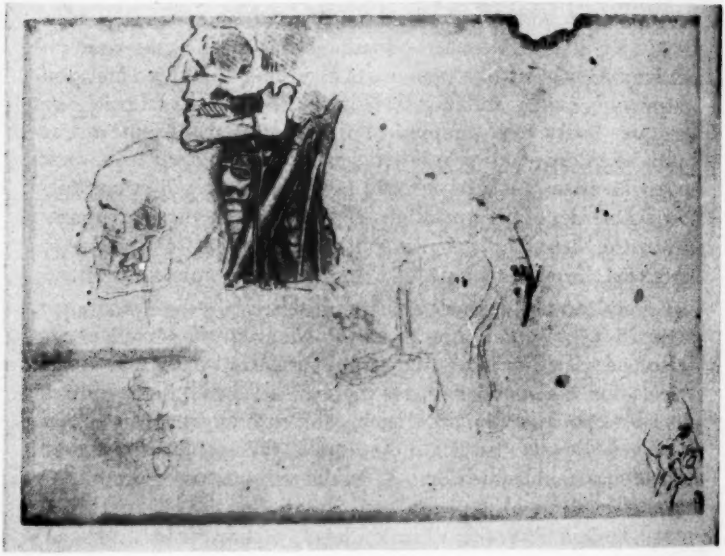
[1] There are three statements available on the number of bodies dissected by Leonardo. Antonio de Beatis, secretary to the Cardinal Louis d' Aragon, reported that on his visit "in October 1517 to Cloux, Leonardo said that" he had dissected more than thirty bodies of men and women of all ages." However, this is a second hand report which also contains so many errors as to be quite unreliable and wholly suspect. The second statement of Leonardo was that "The Pope has known that I have skinned three cadavers." This may refer to his Roman period only, but judging from the manuscripts may not be far from the truth. The third statement given above is ambiguous when taken in context and is probably a generous estimate. One is reminded of Leonardo's contemporary, Berengario da Carpi who stated that he had dissected "over a hundred bodies," obviously as judged by his anatomical writings a gross exaggeration.

shop and it may come as a surprise to many to learn that nearly three-quarters of Leonardo's surviving anatomical drawings depend in whole or in part upon animal dissection.

In this connection, the question naturally arises as to what fraction of Leonardo's anatomical drawings have survived. There are those who contend that the existing anatomical manuscripts represent but a very small fragment of his work. This view is an assumption derived from a statement of Leonardo's in which he discusses the qualities necessary for anatomical investigation and points to his own diligence and lack of time by mentioning "the hundred and twenty books composed by me." [OS 146; QI 13 v] It has rashly been presumed that these books were on anatomy. However, like so many of the remarks in his notes, the statement is highly ambiguous. Nowhere does he specifically say that these books were anatomical and the statement may just as easily be interpreted as referring to his total output as of that time. Of course, this question can never be answered unless further discoveries of his works are made. However, there are many reasons for believing that the existing manuscripts represent a large part, if not the whole, of Leonardo's anatomical output.

The earliest of Leonardo's anatomical drawings are attributed to circa 1487 at which time he was in Milan in the service of the Duke Lodovico Sforza. A study of his drawings from this period reveals that his knowledge was merely such as he had acquired from traditional authorities such as Avicenna and Mundinus, combined with the dissection of animals, the ape, the dog and an ungulate, probably the horse in relationship to his project of the equestrian statue of the Francesco Sforza. So far as his drawings reveal, he had access to a human head, perhaps from an execution, and possibly a human leg. For the most part Leonardo depends for his knowledge of human anatomy upon surface appearances derived from a study of the living subject. Typical of this period is a drawing [OS 34; QV 16 r] of what purports to be a dissection of the human neck in which the larynx is that of an animal, probably the ox, combined with the superficial vessels and muscles which are human but evidently derived from surface inspection. Again in a figure, copied by Albrecht Dürer and to be found in his Dresden Sketch Book [OS 153; QV 21 r], we find the bones of a monkey distorted to the proportions of man. Another figure from this early period is the famous drawing of the cardiovascular system [OS 116; QV 1 r]. The branching of the

arch of the aorta unmistakably identifies its ungulate source which is further indicated by the arrangement of the kidneys as found in animals. The ideas expressed in this figure in which the caval system is observed to arise in the liver, like the sprouting of a seed, indicates the traditional origin of the drawing in Galenical theory. In addition, the astonishing inaccuracies of the superficial veins of the extremities, even though readily open to inspection in the living, reveal a total dependence upon the writings of Mundinus and Avicenna.



OS 34; QV 16 r

This first period in Leonardo's development as an anatomist was not completely barren. His drawings of the skull are exquisite in their detail. Among them is the first representation of the maxillary antrum [OS 4; FB 40 v] which had to await Nathaniel Highmore in 1651 for its full description. However, we should again be reminded that this drawing is no simple morphological illustration but carries with it physiological implications related to the humoral doctrine as the notes reveal. At this time Leonardo introduced the technique of cross-sectional representation and the method of viewing the body from several

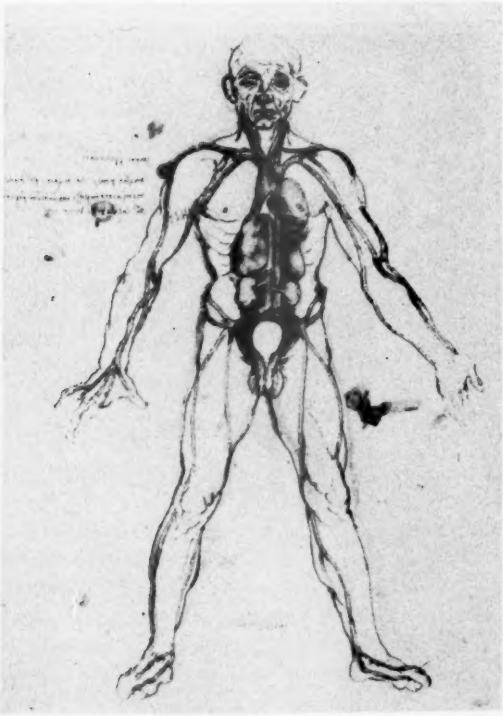
aspects—the later uses of which were to prove so fruitful. Already we see evidence of his inquiring mind although it was certainly not an informed one.

In 1499 Leonardo suffered the greatest of his misfortunes with the loss of his patron Lodovico, il Moro, who had been ignominiously betrayed by his mercenaries at Novara and carried off by Louis XII of France. Thereafter, he was compelled to begin his wanderings which were not to cease until his death at the castle of Cloux. Following a short period of service with the Venetians to advise on their fortifications against the Turkish threat, Leonardo returned to Florence wholly enraptured with the study of mathematics which he continued under the tutelage of his friend, Luca Pacioli. In September of the following year,

OS 153; QV 21 r

1501, Leonardo was in the train of Cesare Borgia as military engineer and proceeded to the conquest of the Romagna and the fall of Camerino and Urbino. Not until March of 1503 was he able to leave the tyrant and enroll once more with the guild of Florentine painters, but almost a year would elapse before things were sufficiently settled to allow of anatomical pursuits.

Leonardo's Florentine period was one of transition. His point of view was gradually changing and his former allegiance to Aristotle was wavering in favour of Galen or, as his contemporaries would have expressed it, he was passing from the ranks of the Philosophers to those of the Physicians, and doubtless this was due to a closer association with the physicians of whom he was beginning to ask questions especially of nomenclature.



OS 116; QV 1 r

It was in this Florentine period that, for the first time, Leonardo seems to have had access to an intact human cadaver. The dissection was carried out on the body of a centenarian who had died in the Hospital of Santa Maria Nuovo, a hospital founded by Folco Portinari, father of Dante's Beatrice. "And I made an anatomy" says Leonardo, "to see the cause of a death so sweet." The series of observations made are among the most brilliant to be found in the notebooks of Leonardo. Here is no dependence on authority, but highly original descriptions and illustrations of arteriosclerosis and of cirrhosis of the liver [OS 128; FB 10 v].

However, side by side with Leonardo's magnificent illustrations and descriptions of his findings in the centenarian are a

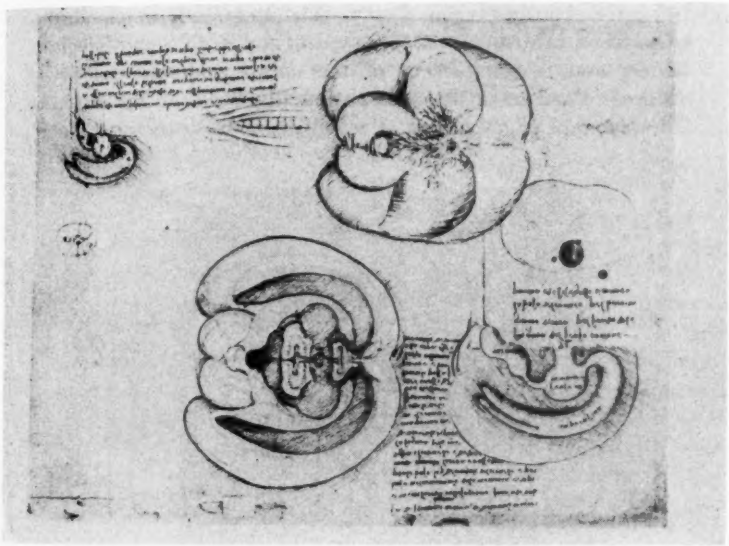
OS 4; FB 40 v

great number of figures in which we recognize distortions of animal anatomy to fit the proportions and outlines of man [OS 140; QV 3 v]. Despite these failings we can only have admiration for the many original techniques which he now began to introduce. These methods exemplify the application of his talents as an artist and as a physicist to the solution of biological problems. Among them is the employment of a solidifying injection mass to determine the shape and extent of the ventricles of the brain; the experiment being carried out on the brain of an ox. In spite of the great difficulites due to the lack of reagents to harden the brain, the results are brilliant [OS 147; QV 7 r]. In addition he

OS 140; QV 3 v

introduced wire models of the muscles for the purpose of analyzing leverage and muscle forces [OS 73; QV 4 r].

Leonardo's second Milanese period began in 1506 under the patronage of the French Regent, Charles d' Amboise. While it is difficult to ascertain precisely in what occupations he was employed by the state, there is evidence to suggest that they were

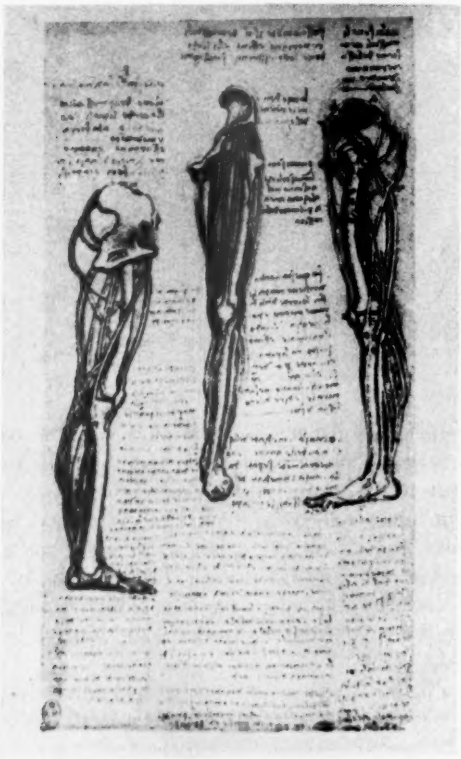


OS 147; QV 7 r

largely engineering projects in relationship to the construction of a canal system. Nevertheless Leonardo appears to have had ample leisure to follow his biological investigations. The representations of human anatomy from this period are among Leonardo's finest, indicating perhaps that he now had greater opportunities for human dissection. However there are many sequential features in these drawings which strongly suggest that for the most part they were derived from the dissection of a single body.

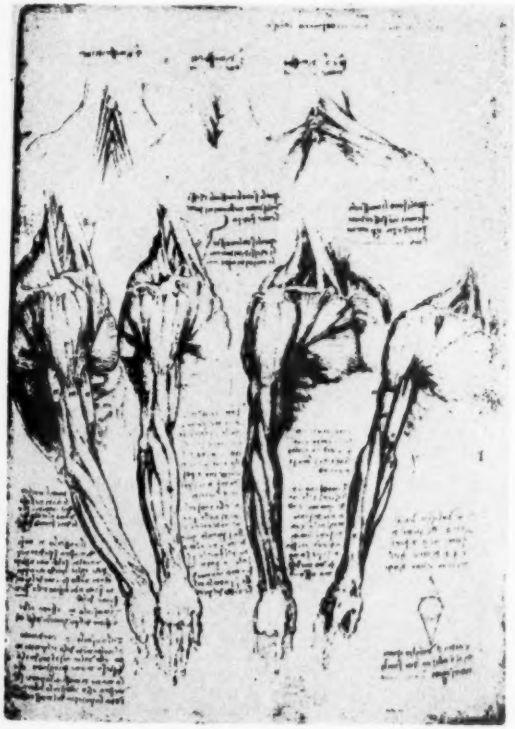
Against a figure such as the superb series on the human upper extremity [OS 46; FA 9 v] we should place one of the most famous of Leonardo's anatomical illustrations, that of the genitourinary system [OS 202; QI 12 r]. Both are from the same period,

circa 1510. The first, exquisite in its accuracy, is undoubtedly human. The second, is just as undoubtedly derived from animal sources distorted to fit the human outline, as is immediately evident in the representation of the heart and arrangement of the great vessels which are characteristic of the ungulate. However, such a figure also clearly demonstrates the inseparability in Leonardo's mind of structure from function and the dominating influence of authoritarian theory. The diagram is an expression in quasi-naturalistic terms of Galenical and Arabist physiological beliefs and is reminiscent of the similar attempt made by Andreas Vesalius in his earlier work, the *Tabulae Sex* of 1538. The presence of hypothetical vessels connecting the uterus and



OS 73; QV 4 r

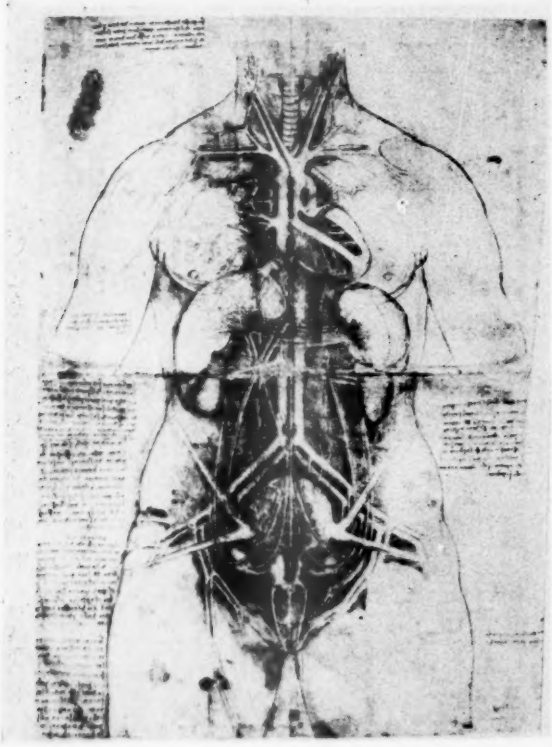
breast give a falsely realistic basis to current notions on the relationship of these organs to menstruation and lactation. The trachea and bronchi, the heart and vessels, the liver and gall bladder, the spleen and splenic vessels, the kidneys and rectal vessels, although naturalistic in form reveal details which show that they are intended to illustrate Galenical theory on the course of the four humours and the formation of the vital and animal spirits. It has not been recognized that in essence such a figure is a curious mixture of human and animal anatomy, humoral doctrine and authoritarian tradition. The figure carries with it the conviction of reality as convincingly portrayed by a great artist but it has in fact little basis in reality and is symbolical of current physiological beliefs.



OS 46; FA 9 v

From the second Milanese period come Leonardo's brilliant enquiries into the mysteries of reproduction, of development and of birth. Here too we might contrast his amazing powers of observation as shown by his representation of the gravid uterus of the cow [OS 211; FB 38 r] with his magnificant figure of the gravid uterus of man in which, however, the placental attachment is derived from the ungulate [OS 210; Q III 8 r]. These figures are magnificent testimony to Leonardo's great powers of observation and scientific acumen, but at the same time reveal his intellectual difficulties.

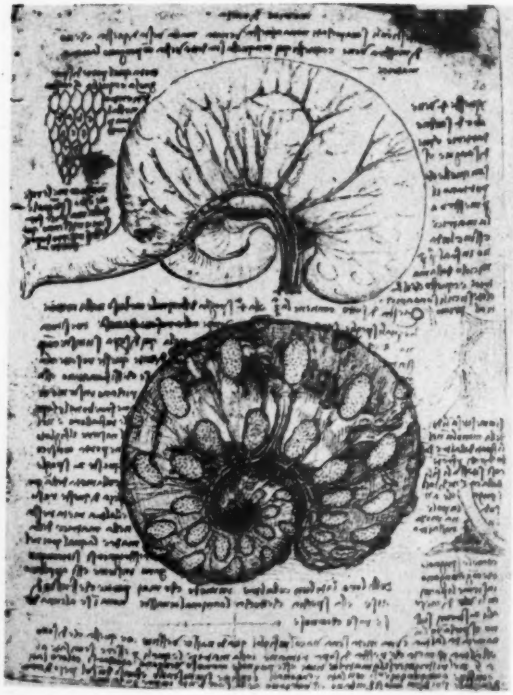
Leonardo's final period as an anatomist began in Rome. The death of Charles d' Amboise in 1511 and of Gaston de Foix before



OS 202; QI 12 r

Ravenna in 1512 discouraged the French in Italy. Milan once more passed into the possession of the Sforza. Leonardo, again without a patron, found employment in the service of Giuliano de Medici, brother of the new Pope, Leo X, and was quartered in the Belvedere. It will be recalled that while in Rome he was denounced to the Pope for his anatomical pursuits by the jealous German mirror-maker and thereafter is said to have given up dissection.

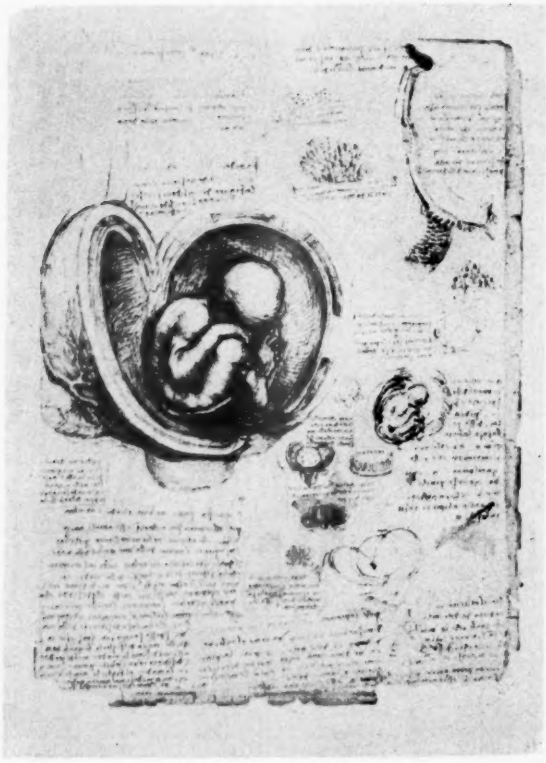
No anatomical drawings can be ascribed to Leonardo's Roman period save the series on the heart. Without exception these studies were conducted on the bovine heart [OS 87; Q II 4 r] which in itself may have been due to Papal prohibition. The series is of great interest and exhibit very great originality of method and independence of thought in the attempt to discern cardiac function. In adopting a quantitative approach to the



OS 211; FB 38 r

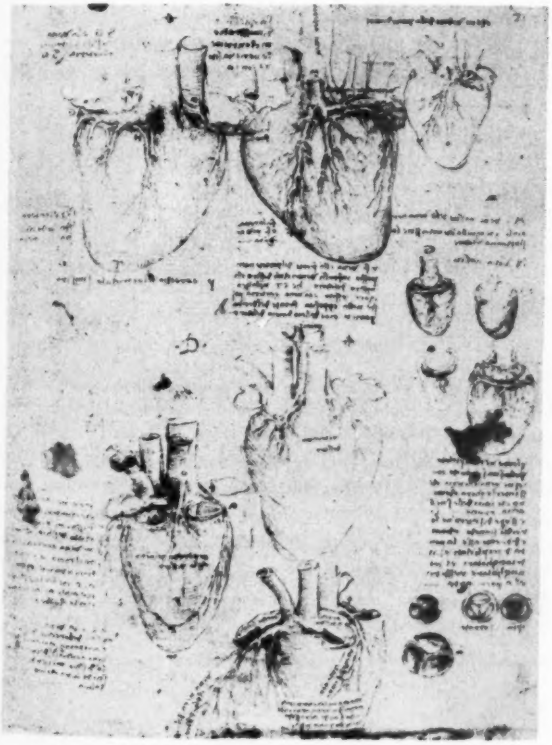
problem of blood volume Leonardo was on the right track but he failed to follow up his own suggestion. In consequence his own views on the circulation were no more than a speculative extension of the Galenical ebb and flow theory. The most important modification was the attempt to give a mechanistic explanation of the ancient mystery of innate heat—an attempt foredoomed to failure but the first blow against vitalism had been struck.

The total contribution of Leonardo to anatomical knowledge when summated in detail is very considerable although his physiological inquiries show no exceptional originality of conception. Not unexpectedly he was guided and directed by the



OS 210; Q III 8 r

stock questions and problems of mediaeval science. He was held in bondage by pre-existing theory, but was essentially right in the manner of his examination and of his testing that theory in light of his own observations. He is magnificent where he attempts to apply quantitative and physical principles to biological problems, but his facts were too few and his physics too slender to support the attempt. He fully appreciated the value of experiment and extraordinarily inventive in suggesting new ones, but his resources were apparently too limited to enable him to carry them out. His most fruitful contribution was the introduction of the graphic arts as a method in an age whose scientists were dominated by adherence to a rigid literary form.



OS 87; Q II 4 r

When we take into account the many difficulties faced by Leonardo in his anatomical and physiological endeavors, the recurring loss of patronage, the distractions of his many interests, the limitations of his resources, and the paucity of his material, we are amazed that he was able to contribute so much.

In understanding Leonardo's difficulties we have a better appreciation of his stature and accomplishment. To call him a comparative anatomist is to lack understanding of the difference between his purely representational drawings of animal anatomy and his desperate attempts to interpret structure in physiological terms. He is greatest in his failure.

Leonardo appears to best advantage as an observer of nature, but he lacked the support of his inheritance to be able to demonstrate the ambiguity of observation alone. The powerful and ingenious, but untrained and unsystematic, intellect of Leonardo de Vinci lies at the crossroads of the accomplishment of man. He is truly a John the Baptist, a forerunner of the science that is to come.

It is commonly held that Leonardo was without influence on the future since his work was never published. This is to ignore the great power of an oral tradition in the early years of the modern era. There is ample evidence from the works of Jerome Cardan, Albrecht Dürer, the Huygens M. S. and other sources to indicate that Leonardo's genius continued to nourish the roots of modern science long after his death.

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CARDIAC ARREST*

A. O. Singleton, Jr., and A. W. De Loach

The term cardiac arrest has come to mean an interruption of cardiac output and not only includes the heart in which no muscle contraction is occurring but the fibrillating heart as well. At the onset we should distinguish cardiac arrest from other critical situations such as cardiac failure in the individual with heart disease, massive pulmonary embolism, cerebral thrombosis, coronary thrombosis, massive hemorrhage, and shock. At our own institution, in reviewing cases coded as cardiac arrest, closer examination revealed other more definite causes of death in many cases. Without attempting to be facetious, we should remind ourselves that the heart stops in every individual who dies and the term cardiac arrest should be used with some discrimination.

Many feel that cardiac standstill either should not have occurred or is an inevitable situation occurring in an individual with cardiac pathology. We shall devote our attention to the cases which are preventable.

Cause of Cardiac Arrest. The usual cause of cardiac arrest is anoxia or more correctly hypoxia. Johnson (1951) believes that this type is the most dangerous with the poorest prognosis. Camp (1953) feels that cardiac arrest will not occur if the patient is in a good state of oxygenation. Anemia, shock or incomplete blood replacement may cause or accentuate a hypoxic state. When the anesthesiologist fits the definition, "an individual half asleep administering an anesthetic to a patient half awake," one can anticipate trouble of this nature. A good airway and proper oxygenation must be maintained.

Giving overdoses of anesthetics and using a multiplicity of agents have been incriminated in many cases. Camp (1953) has attempted to show that multiple anesthetic agents carry a higher mortality rate than the use of a single agent. However, cardiac arrest has been known to occur with every type of anesthetic including local and spinal. Cyclopropane, ethyl chloride, and chlo-

* From the Department of Surgery, The University of Texas Medical Branch, Galveston. Read before the Texas Railway and Traumatic Surgical Association, Fort Worth, Texas, April 25, 1955.

roform are believed to sensitize the heart directly to the action of adrenalin and thus through an increased cardiac excitability bring about cardiac arrest.

Hypersensitivity to anesthetic agents will be an etiological factor in a few cases, and any history of difficulty with preceding anesthetics should be elicited before undergoing an operation.

For many years cardiac standstill, as a result of a vagovagal reflex, was thought to be an important cause. It was felt that any manipulative procedure, such as intratracheal intubation or traction on the gastrointestinal tract, which stimulates afferent vagal fibers might result in a reflex inhibiting of the heart beat. Cowan (1953) believed that this was especially dangerous during light anesthesia. Bonica (1953) reported that one should avoid any unnecessary traction on the heart as this was a factor in the high rate of occurrence in thorasic cases. Atropine has been recommended to block vagal stimulation. More recent evidence tends to minimize vagal effects. Sloan (1950), Stewart (1953) and others studying the problem with animal experimentation found that it was almost impossible to produce cardiac standstill by vagal stimulation alone. Although the inhibiting effects of the vagus on the heart was enhanced by hypoxia, it was necessary to cut off the oxygen supply to the coronary arteries to produce a sudden and permanent cardiac arrest. Lam (1954) tried to stop the heart to see if certain intracardiac operations might be done more easily. He found that vagal stimulation and over-etherization were ineffective in this regard.

In a study of clinical cases Stephenson (1953) felt that a vagal reflex could be incriminated in 156 of 624 cases and felt that atropine would remove this hazard, but others have felt that the evidence for this, even from Stephenson's own work, is rather sketchy. Morton (1953) has stimulated the vagus electrically during operations for non-resectable carcinoma of the lung, but was able to produce no other cardiac disturbance other than a few extra systoles.

Pötts in a series of 750 chest operations in children, most of whom had congenital heart disease, found that the only case resembling cardiac arrest occurred in a very cyanotic child with pulmonary stenosis. Hyperexcitability is thought to be a factor in cardiac arrest according to some authors, but deaths due to excitement usually occur in individuals with previous cardiac disease. Status thymicolymphaticus, once used to explain sudden

death on the operating table in children, is no longer considered to be an etiological entity.

Incidence of Cardiac Arrest. The reported incidence of cardiac arrest has ranged from one to every 1000–5000 anesthetics resulting in 5000–10,000 deaths per year. The first suspected case occurred in 1848. In 1847 Schiff showed the possibility of reviving the arrested heart by massage. While unsuccessful attempts at resuscitation were reported as early as 1889, the first successful case occurred in1901 according to Dale (1952).

In recent years a great deal of interest in this condition has taken place. It is difficult to know whether the incidence is actually increasing, perhaps accentuated by more complex anesthetic and surgical procedures, or whether the increasing number of cases merely reflect the more frequent awareness and recognition of the problem. Cardiac arrest is more common in thorasic operations; 85 per cent of cases occur in the operating room.

Recognition of Cardiac Arrest. Since irreparable damage occurs when the brain is deprived of oxygenated blood from 3–4 minutes, the prompt recognition of the absence of heart function is important. Many times the condition is finally recognized without knowing how long it has existed. This lack of awareness on the anesthetist's part often suggests the etiology of the condition, an anesthetic given in a slovenly manner. The anesthesiologist must be ready to sound the alarm at the absence of a pulse or heart beat. The patient will usually go into respiratory arrest at that time or shortly thereafter. An absence of bleeding, cyanosis of the blood, or absence of pulsation of the major vessels in the surgical field should immediately call the surgeon's attention to a failure of cardiac function.

One must differentiate cardiac arrest from a weak pulse which eludes detection or a temporary stoppage or change of pace which the heart sometimes undergoes on reflex stimulation. Often the correction of the cause, such as an overdose of anesthetic, anoxia, etc. will start the heart to beating, but if arrest can not be ruled out within seconds the chest must be opened and cardiac massage instituted. If blood spurts from the chest wound as the incision is being made, if the patient is conscious, if respiratory effort continues, obviously cardiac stoppage has not occurred. If one is already in the chest, the condition of the heart can readily be ascertained.

Bellville (1955) has advocated the routing use of electroencephalograms during anesthesia, feeling that this is a more sensitive way to recognize hypoxia, depth of anesthesia and hypercapnia than the usual clinical signs.

Treatment of Cardiac Arrest. It is of primary importance in the treatment of cardiac arrest to establish a proper airway, supply oxygen to the lungs and maintain forced respirations. It has been our experience that even a tight fitting mask probably is ineffective and an endotracheal tube must be inserted if this is not already in place. Mouth to mouth breathing may be used until this can be inserted.

Without regard to sepsis or hemostasis, the chest should be rapidly opened in the left fourth interspace. If a mistake is made and one enters the third or fifth interspace no great harm is done however. The incision should extend from the sternum to the left axillary line or further laterally if necessary to obtain good exposure. Though speed is essential, care should be taken not to lacerate the lung as one enters the pleural cavity. One can also usually avoid cutting the internal mammary vessels by starting the incision medially and extending it laterally. The hand is then inserted into the chest, the heart grabbed between the thumb and fingers and a compression of heart started with a flat-handed milking motion. Relaxation should be allowed between compression to allow refilling. Two hand massage may be used in large chests. One should be careful in one's enthusiasm to avoid pushing the thumb into the chambers of the heart. If this occurs, the rent must be closed while massage is continued, a nightmarish task, if there ever was one. Haight (1955) found in his case that the tear could be best closed with a continuous catgut suture as interrupted silk sutures tended to tear out.

The optimum rate of massage is debatable. Some feel that one-half the normal rate is fast enough and a pace that the operators are better able to maintain efficiently. Best, however, feels that a rate of 120 contractions a minute with frequent change of operators to maintain this rate is advisable.

Once oxygenation and massage are established, the critical problem of time is under control. Now it is possible to send for more experienced help and better equipment and carry on other auxiliary procedures. Cutting the 4th and 5th costal cartilages may relieve some of the pressure on the operator's wrist from the ribs. A self-retaining rib-spread adds greatly to the ease of doing

the procedure. Where the abdomen is already open, it is a great temptation to massage the heart subdiaphragmatically or to reach the chest by incising the diaphragm. It is generally agreed that the direct thorasic approach is superior. Some prefer to open the pericardium, others do not.

One must differentiate between cardiac standstill and fibrillation. If the pericardium is opened this can be seen directly, but palpation alone is usually sufficient to make the diagnosis. In fibrillation the heart feels like a bag of worms. If massage doesn't restore the beat in a standstill heart, injection of 1 cc. of 1:1000 of epinephrine solution is recommended. Pricking of the heart with the needle or a blow to the pericardium have been reported to be equally effective. Some prefer not to use epinephrine because of the danger of starting ventricular fibrillation. There are differences of opinion as to which part of the heart is the ideal site for the injection. Kay and Blalock (1951) have recommended the injection of 2–4 cc. of 10 per cent calcium chloride into the left ventricle in cases of persistent standstill. If fibrillation is present, electric shocking of the heart should be done. Commercial defibrillators are available, but cheaper home-made devices have been devised by many authors. Some recommend attaching the two wires of an extension cord to two retractors and applying these to the surface of the heart. A shock is applied by quickly plugging the device into and then quickly out of a plug connecting to the ordinary 110-volt household current. With the commercial defibrillator an electrical stimulus of 110–130 volts of 0.1 second duration is usually given. If one is not enough, 5 to 10 successive stimuli one second apart should be tried. This usually changes the fibrillation to a standstill which can be treated in the usual manner except adrenalin stimulation is avoided.

Quinidine is felt to be useful in these cases by some. Injection of procaine amide into the heart has also been recommended. Swan (1953) feels that potassium chloride is useful in ventricular fibrillation complicating hypothermia for cardiac operations. In some cases, fibrillation has appeared in standstill hearts undergoing massage. Coramine, metranol and caffeine are not helpful in these cases and may do harm. Intra-arterial transfusions have been advocated to help to raise the blood pressure. The Trendlenburg position theoretically should increase blood flow to the brain. Also intermittent clamping of the aorta will help divert

blood to the head. Ivory (1951) has recommended that heparin should be given during the emergency treatment to help prevent small emboli from forming and lodging in the cerebral arterioles. McMillan (1952) has advocated giving nor-epinephrine which would exert a peripheral vaso-motor effect giving an increase in the diastolic blood pressure and yet free of central effects. The need for regular epinephrine would be reduced.

While Zoll (1952) has developed an external electric stimulator which can be used without opening the chest, it would appear that this should be reserved for patients with heart block who are having repeated attacks of asystole and should not be a substitute for cardiac massage in the usual case of cardiac arrest.

The question arises if efforts at resuscitation are unsuccessful, how long should one try to restore the heartbeat. Although Ament (1951) has felt that there is little hope for success if there is no response after 20 minutes, Height (1955) has reported a case of successful resuscitation after three hours and fifteen minutes despite perforation of the heart during massage. Until the last few minutes of massage there was little indication that the attempt at resuscitation would be successful. There was no cerebral damage in his case. Success has also been reported after periods of massage lasting from 53 to 82 minutes. Others have reported prolonged period of effort lasting as long as five hours without any permanent response.

Although the heart action has been restored, if anoxia has existed over four minutes, there is little chance for survival and the individual is left only to a vegetable type of existence. Cerebral edema resulting from the anoxia is believed by some to be a factor in the high mortality in these cases. The use of human serum albumen and 50 per cent glucose intravenously postoperatively has been recommended.

In studying the results of cardiac massage, one finds varying statistical data. The individual or small groups of cases reported are usually the successful ones. In larger series compiled from many sources the author is a long way from his material and many inaccuracies probably are represented. At the Lahey clinic the last fifteen cases reported were all temporarily revived but only seven survived. In a large series of cases collected over the country there were only 250 out of 1,000 who lived. Cole (1951) reports recovery in 112 out of 350 collected cases. Of those surviving, the circulation was restored in less than four minutes in

91 per cent. In cases occurring outside the operating room the results are nearly always fatal.

At the John Sealy Hospital our recovery rate has been very poor, perhaps an understatement since none survived. Although a number of cases have been coded as cardiac arrest, on more careful analysis we have felt that these did not fall into this group. We have eliminated cases who died at or soon after operation where other more obvious causes of death were present. We have not included those cases in which there was a momentary loss of pulse or blood pressure which was restored before any surgical intervention was done. There were seven cases ranging from infancy to old age, involving males and females in relatively equal numbers. Various anesthetic agents were used but cyclopropane was involved in most of the cases. All occurred in the operating room except two; one occurred after operation when the patient was returned to the ward, the other during the reduction of a fracture under general anesthesia in the outpatient department. Two cases occurred during thorasic operations. The others were abdominal with the exception of the fracture case and one during an encephalogram. A cardiac standstill was present in all of the patients. Massage was done through the chest in all cases except one where a transdiaphragmatic approach was done. The heartbeat was restored within ten minutes after starting massage in four cases. In the others no response was obtained though massage was only carried on for about 1 hour. The gamit of cardiac injections was tried in most instances. Although massage was started supposedly in all cases within 4 minutes after cardiac arrest had occurred, the four individuals who had heart action restored never regained consciousness and one must presume that irreparable anoxia of the brain had occurred. Deaths resulted in from four hours to eleven days later. I am led to the conclusion that either cardiac arrest was not recognized at the moment that it occurred, or else a period of anoxia or hypoxia had been present for a prolonged period before the heart stopped beating.

In the three cases where cardiac function was not restored, the question arises as to whether a more prolonged attempt at resuscitation might have been successful. The odds of course are against this.

In conclusion, the main problem in cardiac arrest lies in its prevention and recognition rather than in the active treatment.

Although the condition either should not be allowed to occur or is inevitable due to uncontrollable factors, immediate recognition with energetic treatment will result in the saving of life in some cases.

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CALLING ATTENTION TO:

Items of Possible Interest to Our Friends

August, 1955

This is the last CAT from Galveston. After this, the new address will be Hamilton Hall, Ohio State University, Columbus 10, Ohio. At Ohio State there is developing an intriguing new Health Center under the leadership of Charles Doan. It offers an opportunity for contributive effort again. It is hoped CAT may be continued. For 15 years CAT has been distributed monthly to friends over the world. In many cases it has been the only source of knowing what is going on scientifically & medically in the United States, for American scientific & medical journals are often too expensive to be purchased. It has helped promote scientific friendships, and here let gratitude be expressed for frequent warmth of response. Here also let thanks be given to Miss Mary Jane Steding, who has so faithfully and regularly handled this each month for our friends. So here's to the stimulus of the new venture: let's carry on!

Basic: RK Andjus & Co reanimate frozen rats by diathermy (*J Physiol* 446/541, 547/55). . . . EH Barany & AM Woodin study hyaluronidase in aqueous humor & the hydrodynamic resistance at angle of anterior chamber (*Acta Physiol Scand* 33/257/55). D Bodian reveals emerging concepts of poliomyelitis infection (*Science* 122/105/July 15/55). . . . EG Butler edits *Biological Specificity & Growth* (Princeton Univ. Press, 55, 321pp, $5). . . . PW Dale concludes that operations of Boolean Algebra correspond to operations of nervous system & that this correspondence is not co-incidental (*Psych Quart* 29/45/55). . . . HC Dalton edits symposium on molecular events in differentiation related to cell specificity (*Ann NY Acad Sci* 60/965–1160/55). . . . KAC Elliott, IH Page & JH Quastel edit *Neurochemistry: The Chemical Dynamics of Brain & Nerve* (CC Thomas, Springfield, Ill, 55, 300pp, $12). . . . E Gjone notes blocking action of high doses of thiamine on neuromuscular transmission & on inhibition of cholinesterases, with co-carboxylase (*Acta Pharmacol Toxicol* 11/55, 94/55). . . . H Herzheimer shows 5 hydroxytryptamine causes shock syndrome similar to histamine & acetylcholine, with rapid tolerance, & antagonized by lysergic acid diethyleamide & atropine (*J Physiol* 128/435/55). . . . G Hollinger discusses guanidines & oxidative phosphorylations (*Acta Pharmacol Toxicol* 11/Suppl 1, Munksgaard, Copenhagen, 55, 84pp). . . . JW Jailer edits symposium on hydrocortisone & aldosterone & analogs as therapeutic agents (*Ann NY Acad Sci* 61/281–636/55). . . . R Koch discusses significance of sulfhydryl in radiobiology (*Arzneimittelf* 5/271/55). . . . GJ Martin analyses *Ion Exchange & Adsorption Agents in Medicine* (Little Brown, Boston, 55, 333pp, $7.5). . . . M Rinkel considers enzymatic adrenalin system in development of schizophrenia (*Am J Psychiat* 111/881/55). . . . JA Schneider finds reserpine counteracts show of sham rage in cats & says reserpine inhibits afferent impulses which stimulate sympathetic activity (*Am J Physiol* 181/64/55). . . . RG Schneider & ME Haggard find sickling of red cells is delayed quantitatively until hemoglobin S reaches adult levels around 4 months of age (*Proc Soc Exp Biol Med* 89/

196/55). . . . AJ Stunkard & Co find hyperglycemic glycogenolytic factor ("Glucagon") given IV abolishes hunger & contractions of stomach, with rise in blood glucose (*Ibid* p258). . . . TJ Villalobos & Co find red blood cells & hemoglobin increase with body cooling while white blood cells & platelets disappear (*Ibid* p192). . . . US von Euler & Co report extensive studies on adrenalin & noradrenalin content of tissues & fluids (*Acta Psysiol Scand* 33/Suppl 118, Norstedt, Stockholm, 55, 74pp).

GENERAL: PAG Alfaso & Co tell the story of *La Obstetricia en Venesuela* (Ragon, Caracas, 55, 576pp). . . . AC Benjamin analyses *Operationism*, a critique of PW Bridgman's proposal that all concepts be defined in terms of empirically performable operations (CC Thomas, Springfield, Ill, 55, 154pp, $3.5). . . . Note Editorial suggestions on symbols, conventions, & abbreviations (*Acta Pharmacol Toxicol* 11/1/55). . . . E Garfield proposes citation indexes for science (*Science* 122/108/July 15/55). . . . KD Roeder analyses spontaneous activity and behavior as a psychological problem (*Sci Month* 80/362/55). . . . C Vaille & G Stern survey *Les Stupefiants Fleau Social* (*Expansion Sci*, Francaise, Paris, 55, 244pp). . . . JJ Izquierdo offers *Montana y los Origines del Movimiento Social y Cientifico de Mexico* (*Ciencia*, Mexico DF, 55, 442pp). . . . EA Weinstein & RL Kahn discuss *Denial of Illness: Symbolic & Physiological Aspects* (CC Thomas, Springfield, Ill, 55, 166pp, $4.5).

SEPTEMBER, 1955

IMPORTANT: ES Feuer of Vermont gives brilliant study of *Psychoanalysis and Ethics* (CC Thomas, Springfield, Ill., 55, 134pp. $4), concluding that "goodness" can be psychoanalyzed but not logically defined, & developing the concept that ethics is applied social science.

GENERAL: V Bush also relates science to ethics (*Atlantic Monthly* Aug 55, p 29). B DeVoto chides US Public Health Service on unfair security practices (*Harper's* Aug 55, p 12). . . . RW Gerard well considers biological roots of psychiatry (*Science* 122/225/Aug 5/55. . . . A Gregg pithily tells when to change jobs & why, noting that nothing succeeds like successors & that while everyone gets crowns of thorns the trick is to wear them jauntily over one ear & also that jobs are wisely sought in relation to one's capacities for learning in younger years, doing in middle years, & directing & advising in later years (*Harper's* Aug 55, p 71). . . . F. Guerra & Olivera give useful information on native North American plants used for emotional effects (*Las Plantas Fantasticas de Mexico*, Diario Espanol, Mexico DF, 1954, 122pp). . . . JS Harrar is optimistic about human intelligence & good will solving problems of food for the future (*Science* 122/315/Aug 19/55). . . . Poet-physician M Moore offers a calendar of compassion, *A Doctor's Book of Hours, Including Some Dimensions of the Emotions* (CC Thomas, Springfield, Ill, 1935, 397pp, $7). . . . GD Stoddard, former President Univ Illinois, is sued by AC Ivy, former Vice-President Univ Illinois, for alleged libel in *Krebiozen: The Great Cancer Mystery* (Beacon Press, Boston, 1955, 223pp, $4), after failure of effort to suppress the book (*Science* 122/323/Aug 19/55).

CLINICAL: WE Chamberlain finds cervical air myelography safe & satisfactory (*AMJ Roentg* 74/1/55). . . . CG Curtis & Ethics Council of Alumni Assoc College of Medical Evangelists ask pertly & cleverly *Is Your Halo on Straight?* (San Lucas Press, Los Angeles, 1955, 48pp, $1). . . . P Ermala &

LR Holsti note correlation between clinical incidence of lung cancer & lung localization of tobacco smoke (*Cancer* 8/673/55). . . . F Homburger gives good suggestions on *The Medical Care of the Aged & Chronically Ill*, & features intriguing R Dufy's pen sketches (Little, Brown, Boston/55/253pp, $5.75). . . . JW Patterson suggests cataracts may result directly from insulin lack (*Am J. Physiol* 180/493/55). . . . DC Peete discusses *The Psychosomatic Genesis of Coronary Artery Disease* (CC Thomas, Springfield, Ill, 1955, 240pp, $7.75). . . . H Shay & DCH Sun find triethylene-thio-phosphoramide helpful in inoperable cancer & in leukemia in oral doses 5–10 mgmg daily with blood check (*Cancer* 8/498/512/55). . . . WB Suter finds oral metrazol therapy promising in senility (*Dis Nerv Syst* 16/211/55).

More Basic: Our FA Hartman notes heart weight in birds is greater in species given to strenuous exertion & to life at high altitudes, & is proportionally greater than in mammals (*The Condor* 57/221/55). . . . LF Larionov & Co of Moscow Cancer Institute report anti-tumor action of "sarcolysin," -p-di-2-chlorethylamino-phenyl-alanine (*Lancet* 2/169/July 23/55). . . . PA Shore & Co show reserpine acts by liberating 5-hydroxytryptamine, as shown by increased indole excretion in urine after reserpine administration (*Science* 122/284/Aug 12/55). . . . O Stroume indicates nasal mucosa is important producer of antibodies (*Acta Allergol* 8/251/55). . . . RW Stacy & Co write *Essentials of Biological & Medical Physics* (McGraw-Hill, New York, 1955, 586pp, $10). . . . EL Wynder, according to J Forman, does well in *The Biological Effects of Tobacco, with Emphasis on Clinical & Experimental Aspects* (Little, Brown, Boston, 1955, 215pp, $4.5).

Here's Hoping that this reaches you safely to indicate that we're still on the job, and wishing that all goes well with you, as ever

C. D. Leake

October, 1955

Solid Stuff: GE Anderson describes glucagon as regulator of insulin function (*Science* 122/457/Sept 9/55). . . . GA Boyd details *Autoradiography in Biology & Medicine* (Academic Press, NY, 55, 411pp, $8.8). . . . RL Cooper & AJ Lindsey extract 4 micrograms carcinogen 3,4 benzpyrene from 500 cigarettes (*Brit J Cancer* 9/304/55). . . . EV Cowdry surveys *Cancer Cells* (Saunders, Phila, 55, 718pp, $11). . . . BG Cragg & HNV Temperley make analogy between immediate memory & ferromagnetic hysteresis (*Brain* 78/304/55). . . . S Davson offers comparative study of aqueous humor & cerebrospinal fluid (*J Physiol* 129/111/55). . . . H Eagle lists nutrition needs of mammalian cell in tissue culture (*Science* 122/501/Sept 16/55). . . . JR Elkinton & TS Danowski survey *The Body Fluids* (Williams & Wilkins, Baltimore, 55, 600pp, $10). . . . EC Faust considers *Animal Agents & Vectors of Human Disease* (Lee & Febiger, Phila, 55, 660pp, $9.75). . . . WJ Fry & Co describe ultrasonic lesions in mammalian brains (*Science* 122/517/Sept 16/55). . . . IC Gunsalus & Co describe pathways of carbohydrate metabolism in micro-organisms (*Bact Rev* 19/79/55). . . . SW Hartwell analyses *The Mechanism of Healing in Human Wounds* (CC Thomas, Springfield, Ill, 55, 166pp, $4). . . . M Iida finds glucoside rhodexin (fr leaves of *Rhodea jap*) has cardiotonic action like digitoxin (*Jap J Pharmacol* 4/136, 155/55). . . . J Jurgens describes serum accelerator inactivation principle, -Factor VII-Inhibitor (*Acta*

Haematol 14/57/55). . . . P Karrer reviews curare alkaloids (*Nature* 176/277/Aug 13/55). . . . Y Kobayashi & Co note non-toxic antidiabetic action of Ca mesoxalate (*Jap J Pharmacol* 4/103, 111/55). . . . JA Lovern discusses *The Chemistry of Lipids of Biochemical Significance* (Methuen, London, 55, 144pp, 8s6d). . . . WC Robbins & Co find anti-serum to collagen reduces collagen fibrogenesis (*J Biophys Biochem Cytol* 1/381/55). . . . JR Robinson stimulates in *Reflections on Renal Function* (Blackwell, Oxford, 54, 163pp, 17s6d). . . . RJ Shephard analyses CO_2 balance sheets in heart-diseased & healthy subjects (J Physiol 129/142/55). . . . RW Smith & Co edit symposium on *Hypophyseal Growth Hormone* (McGraw-Hill, NY, 55, 601pp, $12). . . . Our E Von Haam & DG Scarpelli find increased deoxy- & ribonuclleic & positive phoshamidase in cells from benzpyrine induced cervical carcinoma (*Cancer Res* 15/449/55).

CLINICAL: HK Beecher & C Ford review progress of Anesthesis (*Surg Gyn Obs* 101/105–139/55). . . . B Bird recommends careful *Talking With Patients* (Lippincott, Phila, 55, 154pp, $3). . . . EG Gross & MJ Schiffrin epitomize *Clinical Analgetics* (CC Thomas, Springfield, Ill, 55, 101pp, $2.5). . . . H Jolly reports on *Sexual Precocity* (CC Thomas, Springfield, Ill, 55, 267pp, $6.5). . . . L Liebman edits helpful discussion of *Stress Situations* (Lippincott, Phila, 55, 144pp, $3). . . . WE Oates studies *Religious Factors in Mental Illness* (Associated Press, New York, 55, 161pp, $3.5). . . . D O'Neill considers emotional factors between *Doctor and Patient* (Lippincott, Phila, 55, 197pp, $5). . . . G Roheim intrigues in *Magic and Schizophrenia* (International University Press, New York, 55, 230pp, $4.5). . . . CS Russell ably discusses "proof" in clinical research (*Lancet* 2/335/Aug 13/55). . . . Sir Lionel Whitby well describes evolution of treatment of Addisonian anemia (*Brit Med J* 1/1401/June 11/55).

GENERAL: F Cottrell discusses *Energy & Society* (McGraw-Hill, NY, 55, 330pp, $6). . . . M Hachiya, physician, reveals his *Hiroshima Diary* (Univ North Carolina Press, Chapel Hill, 55, 176pp, $3.5). . . . M Mead & Wolfenstein edit *Childhood in Contemporary Cultures* (Univ. Chicago Press, Chicago, Ill, 55, 502pp, $7.5). . . . M Natenberg is iconoclastic in *The Case History of Sigmund Freud* (Regent House, Chicago, Ill, 55, 188pp, $4). . . . R Pabel concludes *Enemies Are Human* (Winston, Phila, 55, 210pp, $3.5). . . . GR Price well criticizes extra-sensory perception & parapsychology reports, deploring scientific flirtation with supernatural (*Science* 122/359/Aug 26/55). . . . WC Rappleye & Co review broad activities of *The Josiah Macy Jr Foundation 1930–1955* (Josiah Macy Fdation, 16 W 46, NYC, 55, 174pp). . . . Note good Symposium on medical libraries in relation to medical education (*J Med Educ* 30/489–532/55). . . . Lee T'ao describes Chinese medicine during Chin (1127–1234) & Yuan (1234–1368) eras (*Chinese Med J* 73/241/55). . . . F Thistlewaite ably discusses *The Great Experiment* (of US culture) (Cambridge Univ. Press, NYC, 55, 321pp, $5).

TEXAS REPORTS ON BIOLOGY AND MEDICINE

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